International congress on transposable elements

2024

Abstract book

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Important information

Welcome to the International Congress on Transposable Elements (ICTE). Below you will find important information for your stay in Saint-Malo, and to fully enjoy the conference. If you have any questions, the organizing committee is available at icte2024@sciencesconf.org and can be quickly spotted during the conference thanks to their gray badge lanyards.

Wi-Fi Login: ICTE2024 Psw: #icte2024!

Opening conference: be ready to connect to https://app.wooclap.com/ICTE2024 with your phone or computer as we will interact and get to know each other before our four-day conference marathon.

Speakers: upload your talks before your session, either early in the morning or during the breaks. Please, respect the timing. To facilitate interactions, you will be visible during the conference thanks to your white badge lanyard.

Chairs: show no mercy for speakers running too long.

Social media: we are present at twitter #ICTE2024 or you can tag us with our handle @ICTE2024. You can find us at Bluesky by using the keywords "TEworldwide" along with all TE-related posts. If a talk has the "no social media", "no twitter" logo, please respect it and do not share the content presented.

Gala dinner (Tuesday evening, April 23): in order to avoid spoiling food and to facilitate the organization of the gala dinner, please tell us as soon as possible if you cannot attend this dinner by writing an email to icte2024@sciencesconf.org.

PhD and post-doctoral positions: Thanks to Ilya Kirov, you can post or search for TE-related PhD and post-doctoral positions here: https://docs.google.com/document/d/1oRGliWkKCTY-8oh94Jp-VMDXhqCI689-2C8XHtRBvco/edit?usp=sharing

Tourism: the Palais du Grand Large has a very good tourist information page https://www.pgl-congres.com/en/pourquoi-choisir-saint-malo/tourisme-saint-malo/

We hope you will enjoy the conference, be amazed by the science and build new interactions for your next transpositions.

Kenavo,
The organizing committee

Schedule

SATURDAY, APRIL 20

4:00 – 6:30 pm	Registration
6:30 pm	Welcome address Organiser: Gael Cristofari, University Côte d'Azur, FR
7:00 – 8:00 pm	**EMBO member keynote lecture** Chair: Gael Cristofari, University Côte d'Azur, FR
7:00 pm	**Keynote lecture 1: Richard Durbin, University of Cambridge, UK** *New ways to look at transposons in genome sequences from across the tree of life*
8:00 pm	**Welcome reception at Palais du Grand Large**
11:00 pm	Closing doors

SUNDAY, APRIL 21

Session I: TEs, genome evolution and adaptation (1/2)
Chair: Alexander Suh, Leibniz Institute for the Analysis of Biodiversity Change, DE

9:00 am	**Cedric Feschotte**, Cornell University, US *Transposon Addiction*
9:30 am	Selected talk: Tanay Ghosh, Altos Lab, UK *RetroMyelin: a retroviral role in the emergence of myelin*
9:50 am	Selected talk: Maya Voichek, Institute of Molecular Biotechnology, AT *Learning new infectivity tricks from old endogenous retroviruses*
10:10 am	**Coffee break**
10:40 am	**Christine Beck**, The Jackson Laboratory, US *Repetitive sequences generate genomic change*
11:10 am	Selected talk: Cécile Courret, Paris-Saclay University, FR *Rapid turnover of centromeric DNA reveals signatures of genetic conflict in Drosophila*
11:30 am	Selected talk: Alejandro Burga, Institute of Molecular Biotechnology, AT *How do genes venture into the unknown? Mavericks—a new vector of horizontal gene transfer in animals*

11:50 am **Flash talks (Poster session 1: 4 selected)**
Chair: Rita Rebollo, INRAE, FR

1. Manvendra Singh - *Co-opted endogenous retrovirus governed transcriptional and post-transcriptional regulation of Pregnancy Specific Glycoprotein 9 (PSG9) locus in the development of healthy and preeclamptic placentation*
2. Brice Letcher - *A role for transposons in the evolution of programmed DNA elimination in* Mesorhabditis nematodes
3. Miriam Merenciano: *Contribution of transposable elements in the sex gap longevity of different* Drosophila *species*
4. Erin Kelleher: *Bruno is a host co-factor that establishes natural variation in P-element transposition*

12:05 pm Lunch at Palais du Grand Large

Session I: TEs, genome evolution and adaptation (2/2)
Chair: Kirsten Senti, Institute of Molecular Biotechnology, AT

2:00 pm **Josefa Gonzalez**, Institute of Evolutionary Biology, ES
Transposable elements contribution to intraspecies adaptation and interspecies evolution in Drosophila and Anopheles

2:30 pm **Harmit Malik**, Fred Hutchinson Cancer Research Center, US
Evolutionary arms races between host and viral genomes

3:00 pm Selected talk: Russell Corbett-Detig, University of California Santa Cruz, US
Horizontal transmission of diverse transposable elements generates introns across the eukaryotic tree of life

3:20 pm Selected talk: Irina Arkhipova, Marine Biological Laboratory, US
Reverse Transcriptase-Related Genes at the Intersection of Environmental Stress Response Pathways

3:40 pm Coffee break

Session II: TE in health and disease (1/2)
Chair: Rebecca Berrens, University of Oxford, UK

4:00 pm **Geoff Faulkner**, University of Queensland, AU
Long-read nanopore mobile DNA methylomics

4:30 pm Selected talk: Vivien Horvath, Lund University, SE
Local heterochromatin mitigates the cis-regulatory impact of SVA transposons in human brain development and disease

4:50 pm Selected talk: Victoria Belancio, Tulane University, US
Assessment of L1 retrotransposition in DNA repair deficient transgenic mouse models

5:10 – 7:50 pm Poster session 1

8:00 pm Closing doors, Dinner on your own

MONDAY, APRIL 22

Session II: TE in health and disease (2/2)
Chair: Prescott Deininger, Tulane University

9:00 am **John Sedivy**, Brown University, US
The aging repeatome: connection to chronic inflammation

9:30 am **Carla Saleh**, Institut Pasteur, FR
Role of retrotransposons in insect antiviral immunity

10:00 am Selected talk: Ana Ariza-Cosano, University of Granada, ES
Endogenous Retrovirus-derived RNAs play an essential role during morphogenesis in zebrafish

10:20 am Coffee break

10:50 am **Kathy Burns**, Dana-Farber Cancer Institute, US
Retrotransposon in cancer: the Marker and the Mutator

11:20 am **Josh Dubnau**, Stony Brook University, US
Intercellular propagation of neurodegeneration by endogenous retroviruses

11:50 am Selected talk: Julia Fuchs, Collège de France, FR
Nuclear translocation of LINE-1 encoded ORF1p alters nuclear envelope integrity in human neurons

12:10 pm Flash talks (Poster session 2: 4 selected)
Chair: Clémentine Vitte, Paris-Saclay University, FR

5. Florian Full - *DNA virus infections shape transposable element activity in vitro and in vivo*
6. Rocio Enriquez-Gasca - *The role of LINE-1 elements in the induction of type I interferon following epigenetic dysregulation*
7. Charlotte Proudhon - *Non-invasive multi-cancer diagnosis using DNA hypomethylation of LINE-1 retrotransposons*
8. Pierre Bourguet - *CDCA7: A Key Contributor to Transposon DNA Methylation in Natural* Arabidopsis *Populations*

12:35 – 3:30 pm Lunch on your own and free time

3:30 – 6:00 pm Poster session 2

6:00 – 7:00 pm Keynote lecture
Chair: Pascale Lesage, University Paris Cité, FR

6:00 pm Keynote lecture 2: Sandra Duharcourt, Institut Jacques Monod, FR
Programmed genome elimination in Paramecium, a definitive form of transposon silencing

Session III: TE control and epigenetics (1/3)
Chair: Andrea Schorn, Cold Spring Harbor Laboratory, US

7:00 pm Selected talk: Abdou Akkouche, University Clermont Auvergne, FR

	A dual histone code specifies the binding of heterochromatin protein Rhino to a subset of piRNA source loci
7:20 pm	Selected talk: Maria Ninova, University of California Riverside, US *SUMOylation "hot spots" in piRNA-mediated TE silencing pathways revealed by diGly proteomics*
7:40 pm	Closing doors, Dinner on your own

TUESDAY, APRIL 23

Session III: TE control and epigenetics (2/3)
Chair: Laure Teysset, Sorbonne Université, FR

9:00 am	**Rob Martienssen**, Cold Spring Harbor Laboratory, US *Retrotransposon addiction underlies centromere function via chromatin remodeling and RNA interference*
9:30 am	Selected talk: Marie Fablet, University of Lyon, FR *Transposable elements and antiviral immunity*
09:50 am	Selected talk: Ronald Van Rij, Radboud University, NL *The piRNA pathway mediates transcriptional silencing of LTR-retrotransposons in the soma and germline of Aedes mosquitoes*

10:10 am Coffee break

Session III: TE control and epigenetics (3/3)
Chair: Helen Rowe, Queen Mary University of London, UK

10:40 am	**Deborah Bourc'his**, Institut Curie, FR *A new spin to chromatin regulation of retrotransposons*
11:10 am	Selected talk: Alex de Mendoza, Queen Mary University of London, UK *DNA methylation enables recurrent endogenization of giant viruses in a protist closely related to animals*
11:30 am	Selected talk: Sophie Lanciano, University Côte d'Azur, FR *Locus-level L1 DNA methylation profiling reveals the epigenetic and transcriptional interplay between L1s and their integration sites.*
11:50 pm	Selected talk: Leandro Quadrana, Paris-Saclay University, FR *Fighting back epigenetic control: A story about the evolution and function of transposon-encoded anti-silencing systems*

12:10 pm Lunch

Session IV: Transposition mechanisms and applications (1/2)
Chair: Didier Mazel, Institut Pasteur, FR

2:00 pm	**Sam Stenberg**, Columbia University, US *Molecular innovation at the interface between CRISPRs and transposons*
2:30 pm	**Orsolya Barabas**, University of Geneva, CH

	The inner workings of DNA transposition assemblies
3:00 pm	Selected talk: Mireille Bétermier, Paris-Saclay University, FR *A developmental condensin I complex assists the Paramecium PiggyMac domesticated transposase to carry out programmed DNA elimination*
3:20 pm	**Coffee break**
3:40 pm	**Jose Penades**, Imperial College London, UK *Redefining mobility in bacterial genetics*
4:10 pm	Selected talk: Baptiste Darracq, Institut Pasteur, FR *Integron cassettes commonly integrate into bacterial genomes via widespread non-classical attG sites*
4:30 pm	Selected talk: Marie Mirouze, IRD, FR *Plant genome stability and extrachromosomal circular DNA (eccDNA): vicious or virtuous circle?*
4:50 pm	Coffee break
Session IV: Transposition mechanisms and applications (2/2) Chair: Kasia Siudeja, Paris-Saclay University, FR	
5:10 pm	**Severine Chambeyron**, Institute of Human Genetics, FR *Impact of retrotransposons on the Drosophila genome*
5:40 pm	Selected talk: Martin Taylor, Harvard Medical School, US *Structures, Functions, and Adaptations of the Human LINE-1 ORF2 Protein*
6:00 pm	Selected talk: Akanksha Thawani, University of California Berkeley, US *Genetic Architects: How the LINE-1 Transposon Crafts a Third of Your Genome*
6:20 pm	**Conclusion and Award announcement** Organisers: Emilie Brasset, University Clermont Auvergne, FR ; Clément Gilbert, Paris-Saclay University, FR
7:15 pm	**Cocktail at Palais du Grand Large**
8 pm	**Gala Dinner at Palais du Grand Large**
12 am	Closing doors

WEDNESDAY, APRIL 24

9 am – 1 pm	**TE Hub satellite workshop** Organisers: Clément Goubert, McGill University, CA; Johann Confais, INRAE, FR
1:00 pm	Closing doors

Abstracts

Oral presentations in order of appearance

EMBO member keynote lecture

New ways to look at transposons in genome sequences from across the tree of life

Richard Durbin

University of Cambridge, UK

Abstract

Due to sustained advances in long read sequencing in recent years, we are now obtaining essentially complete, high contiguity chromosomal assemblies at an increasing rate and decreasing cost. More than 1,500 new species reference genome sequences have been completed within the Tree of Life programme at the Wellcome Sanger Institute, and other large scale projects such as the Vertebrate Genomes Project and European Reference Genome Atlas are also scaling up. Because these assemblies contain both haplotypes in diploids (more in polyploids), they reveal heterozygous genomic insertions, and this provides a new basis for identifying and annotating recently active transposable element families – see also the poster on new software Pantera from Pio Sierra. Using Pantera we have investigated the evolution of large, low copy number transposon families such as Mavericks/Polintons across hundreds of species. Further, in most cases even heterochromatic repeat DNA is well assembled, so we have been able to characterise turnover of centromere-associated repeats which are some of the most rapidly evolving seqence in the genome, observing repeated transitions in plants between satellite tandem repeats and transposon “nests” formed by serial invasion of LTR retrotransposon families.

Session I: TEs, genome evolution and adaptation

Transposon Addiction

Cédric Feschotte
Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA

Abstract

Over the past decade it has become clear that transposable elements make up the bulk of eukaryotic genomes and have had a profound impact on the biology and evolution of species. Notably, examples now abound of TE sequences coopted to assemble new genes, regulate gene expression and fuel evolutionary novelty. However, in nearly all cases of TE cooption, only parts of individual TE sequences have been repurposed for cellular function and the coopted TE has long ceased to be capable of transposition. Thus, the dogma remains that, when transpositionally active, TEs are best viewed as selfish genetic elements providing no direct benefit to the host and even threatening genome integrity. In this talk, I will challenge this dogma and present evidence that active TE families can engage in activities indispensable for organismal development. We focus on two families of endogenous retroviruses in zebrafish called Bhikhari (Bik-1 and Bik-2) with nearly identical and insertionally polymorphic copies suggesting that both have been transpositionally active in the germline very recently. Interestingly, both families have evolved distinct expression patterns during embryonic development: Bik-1 is specifically expressed in the mesoderm lineage while Bik-2 (also known as crestin) marks the neural crest lineage. Manipulative experiments in zebrafish embryos demonstrate that Bik-1 and Bik-2 express Gag proteins essential for proper mesoderm and neural crest development, respectively. Mechanistically, it appears that Bik-1 Gag associates with membrane and promotes cell adhesion and/or oppose cell migration in a cell-autonomous fashion. Moreover, yet another endogenous retrovirus in chicken, ERNI, encodes a Gag protein regulating neural crest cell development in this species. Thus, on at least three separate occasions, the Gag proteins encoded by active TEs have become required for vertebrate development. We propose that such interactions arise progressively during evolution as a form of addiction to TE products, in this case Gag proteins. We hypothesize that TE products introduce cellular activities that are redundant with and occasionally displace host-encoded products essential for embryonic development, such as those controlling cell adhesion/migration. Such functional displacement creates a dependency on the TE product for host development, which in turn promotes the maintenance but limit the propagation of the TE in the germline. These findings support the provocative idea that TE activities are inextricably intertwined with organismal development, as envisioned by Barbara McClintock but largely dismissed for more than half a century.

RetroMyelin: a retroviral role in the emergence of myelin

Tanay Ghosh*[1,2,3], Rafael Almeida[4], Chao Zhao[1,2,3], Abdelkrim Mannioui[5], Elodie Martin[6], Alex Fleet[2,3], Civia Chen[1,2,3], Peggy Assinck[1,2,3], Sophie Ellams[1], Ginez Gonzalez[2,3], Stephen Graham[7], David Rowitch[2,8], Katherine Stott[9], Ian Adams[10], Bernard Zalc[6], Nick Goldman[11], David Lyons[4], and Robin Franklin[1,2,3]

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[3] Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom
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[8] University of Cambridge – Department of Paediatrics, United Kingdom
[9] University of Cambridge – Department of Biochemistry, United Kingdom
[10] MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, United Kingdom
[11] European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, United Kingdom

Abstract

The evolutionary appearance of myelin (the lipid rich sheath surrounding axons) in vertebrate bestowed a spectrum of selective advantages: it facilitates accelerated nerve impulse propagation allowing complex brain structures and enhanced morphological diversity. Our research has unveiled a critical link between myelination and the RNA level expression of RNLTR12-int, a retrotransposon of retroviral origin, demonstrating its indispensable role in this process. We have identified a mechanism whereby SOX10 binding to this RNA regulates transcription of myelin basic protein (Mbp, the major constituent of myelin) in rodents. Furthermore, global analysis of gene expression revealed that inhibition of RNLTR12-int specifically perturbed myelination network. The temporal coincidence of myelin emergence with vertebrate jaw development is striking; jawless vertebrates notably lack both Mbp and compacted myelin. We identified RNLTR12int like sequences (which we termed RetoMyelin) in all jawed vertebrates and demonstrated their functions in two disparate vertebrate classes (fish and frogs). Our study suggests that the acquisition of these sequences in species likely occurred through convergent evolution and co-opted for myelination function, positing retroviral endogenization is a seminal event in the origin of myelin.

Keywords: Retrotransposable element, convergent evolution, cooption, Myelin, Gene regulation, Oligodendrocytes, Neuroscience

* Speaker

Learning new infectivity tricks from old endogenous retroviruses

Maya Voichek*[1], Kirsten-André Senti[1], and Julius Brennecke[1]

[1] Institute of Molecular Biotechnology – Dr. Bohr-Gasse 3, 1030 Vienna, Austria, Austria

Abstract

Endogenous retroviruses are abundantly embedded within host genomes and provide a unique snapshot of multiple past viral infections. They are thought to be evolutionarily related and structurally similar to LTR retrotransposons, yet retroviruses primarily differ by a canonical fusogenic Envelope protein crucial for cell-cell infectivity. We have discovered a group of active transposons in the *Drosophila* ovary that mimic retroviral behavior demonstrating infectivity traits - despite the absence of an Envelope-coding gene. We further identified an alternative infectivity gene encoded in the genomes of these transposons, potentially substituting the Envelope's role in enabling cell-cell transmission. By mining genomes, we found such infectivity genes to be widespread in the context of transposons across insects. These findings, which call attention to the concepts of infectious transposons or Envelope-less retroviruses, necessitate reconsideration and redefinition of the conventional boundaries between transposons and viruses.

Keywords: Endogenous retroviruses, LTR retrotransposons, Drosophila, Infectivity, Envelope

* Speaker

Repetitive sequences generate genomic change

Parithi Balachandran[1], Weichen Zhou[2], Mark Loftus[3], Bernardo Rodriguez-Martin[4], Peter A. Audano[1], Yanming Gan[5], Jan O. Korbel[6], Ryan E. Mills[2], Miriam K. Konkel[3], **Christine R. Beck[1,7]**

1. The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA, **2.** University of Michigan Medical School, Ann Arbor, MI, USA, **3.** Clemson University, Clemson, SC, USA, **4.** Centre for Genomic Regulation, Barcelona, Spain, **5.** Harvard University, Cambridge, MA, USA, **6.** European Molecular Biology Laboratory, Heidelberg, Germany, **7.** UConn Health Center, Farmington, CT, USA

Abstract

Transposable elements (TEs) diversify human genomes through retrotransposition, polymorphism, and recombination. Polymorphic TE Insertions (TEIs) contribute to both inter- and intra-individual genetic variation, leading to ongoing mutagenesis and disease. Further, two homologous TEs can act as substrates of ectopic DNA repair leading to different types of structural variants. In this study, we have conducted a thorough analysis of TE-driven variation across sequence-resolved assemblies of 64 phased human haplotypes from diverse individuals.

In these 64 haplotypes, we identified 9,388 non-redundant polymorphic TEIs, including 1,145 LINE-1s, 7,609 *Alu* elements, 532 SVAs, 21 HERV-Ks, and 31 processed pseudogenes (PPGs) comprising 7.4 Mbp of sequence. The majority (64%) of these insertions fell within existing repetitive regions, where we observed nested 'repeats in repeats', i.e. *Alu* insertions within older *Alus* and LINE-1s inserted within LINE-1s. Additionally, we identified 2,417 reference TEs, including 162 LINE-1s, 2,165 *Alu* elements, 86 SVAs, 1 HERV-K, and 3 PPGs that exhibited polymorphic deletions, indicating that individuals in our study lacked these reference insertions.

Our assembly data have also enabled the interrogation of allelic heterogeneity associated with each sequence-resolved TEI locus. For 674 reference and polymorphic full-length human-specific LINE-1 sequences, we have cataloged the extent of allelic heterogeneity at each locus across the 64 haplotypes. We have found that heterogeneity positively correlated with insertion allele frequency. Notably, allelic differences frequently impact the coding and mobilization potential for LINE-1s, including hot (i.e. highly active) elements.

In addition to insertion-driven variation, we have also identified 1,634 *Alu*-*Alu*, 472 LINE-1-LINE-1 and 3 SVA-SVA mediated rearrangements that affect nearly 12 Mbp of human DNA. *Alu* elements drive 79% of TE-Mediated Rearrangements (TEMRs) and more than half of them occur within genes. We further inspected TE-mediated inversions and found small deletions or duplications at the junctions of 75% of *Alu* driven and 9% of LINE-1 driven TEMRs, indicating the role of replication-based mechanisms.

In summary, sequence-resolved genomic assemblies of diverse individuals have enabled a comprehensive understanding of transposable element-derived variation between genetically diverse individuals, highlighting extensive differences caused by transposon mobility, allelic heterogeneity, and TE-driven rearrangements.

Rapid turnover of centromeric DNA reveals signatures of genetic conflict in Drosophila

Cécile Courret*[1], Lucas Hemmer[1], Xiaolu Wei[1], Prachi Patel[2], Bryce Santinello[2], Xuewen Geng[1], Ching-Ho Chang[3], Barbara Mellone[2], and Amanda Larracuente[1]

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2 Department of Molecular and Cell Biology, University of Connecticut – Storrs, Connecticut, United States
3 Division of Basic Sciences, Fred Hutchinson Cancer Center – Seattle, Washington, US, United States

Abstract

Centromeres are chromosomal structures required for faithful genome inheritance during cell division. Centromeres are defined epigenetically by the presence of the centromerespecific histone H3 variant, CENP-A. While centromeres form in repeat-rich regions of the genome, the roles of DNA sequences in centromere function are unclear. We recently revealed that all centromeres in *D. melanogaster* correspond to islands of complex DNA enriched in retroelements and flanked by tandem repeats. Each centromere is unique-the only sequence they have in common is the *G2/Jockey-3* retroelement. It is unclear if any of these sequences are important for centromere function. Here we study the evolution of centromere composition to gain insights into the role of DNA sequence in centromere biology. We combined (epi)genomic and cytological approaches to characterize centromere organization in three sister species: *D. simulans, D. sechellia,* and *D. mauritiana*. We discovered dramatic centromere reorganization involving recurrent shifts between retroelements and satellite DNAs over short evolutionary timescales (< 240 Kya). None of the *D. melanogaster* centromere islands are conserved in the *simulans* clade. Instead, in the *simulans* clade centromeres are mainly composed of two complex satellites: *500bp* and *365bp*. Those two complex satellites are specific to the *simulans* clade, suggesting that they invaded and replaced centromeres after the split with *D. melanogaster*. In addition, we observed a second replacement event specific to *D. sechellia*, where the dot and X chromosome centromeres now sit on telomerespecific retroelements, revealing for the first time true telocentric chromosomes. Finally, *G2/Jockey-3* is enriched in *D. simulans* centromeres, but much less so in *D. sechellia* and *D. mauritiana.* Identifying the functional centromeric DNA shed new light into their roles in chromosome function and evolution. Our results highlight the very rapid turnover of centromeric sequences among the *melanogaster* clade and are consistent with recurrent genetic conflict.

Keywords: centromere, genetic conflict, Drosophila, repeat sequences

* Speaker

How do genes venture into the unknown? Mavericks-a new vector of horizontal gene transfer in animals

Sonya Widen , Israel Campo Bes , Alevtina Koreshova , Pinelopi Pliota , Daniel Krogull , and Alejandro Burga*[1]

[1] IMBA – Dr. Bohr-Gasse 3, Azerbaijan

Abstract

Horizontal gene transfer (HGT)-the movement of genetic material between species-has been reported across all major eukaryotic lineages. However, the underlying mechanisms of transfer and their impact on genome evolution are still poorly understood. While studying the evolutionary origin of a selfish element in the nematode *C. briggsae*, we discovered that *Mavericks,* ancient virus-like transposons related to giant viruses and virophages, are one of the long-sought vectors of horizontal gene transfer. *Mavericks*-also known as *Polintons*-are found in almost every major eukaryotic lineage. They are flanked by terminal inverted repeats and can readily jump and insert into genomes, like transposons. But like viruses, they code for a large number of proteins, including a type-B DNA polymerase, a retroviral-like integrase, as well as major and minor capsid proteins. Using a combination of phylogenetics, structural predictions and genetic crosses, we discovered that two novel nematode gene families-*wosp* proteases and *krma* kinases-are preferentially taken up as cargo genes by *Mavericks* and have been extensively transferred between different nematode species on a global scale. We also found that nematode *Mavericks* captured a novel fusogen, MFUS-1, which is structurally similar to the glycoprotein B from *Herpes simplex virus 1.* This event likely fueled their spread via the formation of enveloped infective particles, analogous to the inception of retroviruses from genomic retroelements. Lastly, we show how the union between a horizontally transferred *wosp* protease, *msft-1*, and a MULE transposon gave birth to a novel class of selfish gene in *C. briggsae*: a mobile toxin-antidote element that causes genetic incompatibilities that drive in wild populations. Our results identify the first wide-spread vector of HGT in animals and highlight how the intertwined biology of viruses and transposons can ultimately impact gene flow between populations, shaping the evolution of the species that carry them.

Keywords: horizontal gene transfer, maverick, polinton, giant transposon, genetic incompatiblities, selfish genetic element, nematode, virus, like, fusogen

* Speaker

sciencesconf.org:icte2024:529609

Transposable elements contribution to intraspecies adaptation and interspecies evolution in Drosophila and Anopheles

Josefa González

Institute of Evolutionary Biology, CSIC.

Abstract

How organisms adapt to the environment is still an open question in Biology. Whole-genome short read sequencing has allowed to explore the role of single nucleotide polymorphisms (SNPs) in environmental adaptation. However, SNPs alone can only explain a fraction of the existing phenotypic variation that is ecologically relevant. Among the structural variants that can now be studied, thanks to the availability of long-read sequencing, transposable elements are likely to play a major role in adaptation due to their capacity to generate mutations that often have phenotypic effects of a complexity that is not achievable by a single point mutation. In our lab, we are investigating the contribution of transposable elements to environmental adaptation in *Drosophila* species adapted to different environments and in *Anopheles* species adapted to urban environments. To facilitate the *de novo* TE annotation in these and other species, we have developed a computational pipeline, MCHelper, that facilitates the curation of TE libraries.

Fitness landscapes of host-virus evolutionary arms-races

Harmit S. Malik

Fred Hutchinson Cancer Center, Seattle, WA, USA

Abstract

Host antiviral proteins engage in evolutionary arms races with viruses, in which both sides rapidly evolve at interaction interfaces to gain or evade immune defense. For example, primate TRIM5α uses its rapidly evolving 'v1' loop to bind retroviral capsids, and single mutations in this loop can dramatically improve retroviral restriction. However, it is unknown whether such gains of viral restriction are rare, or if they incur loss of pre-existing function against other viruses. Using deep mutational scanning, we comprehensively measured how single missense mutations in the TRIM5α v1 loop affect restriction of divergent retroviruses including HIV-1. Unexpectedly, we found that the majority of mutations increase weak antiviral function. Moreover, most random mutations do not disrupt potent viral restriction, even when it is newly acquired via a single adaptive substitution. Our results indicate that TRIM5α's adaptive landscape is remarkably broad and mutationally resilient, maximizing its chances of success in evolutionary arms races with retroviruses. In contrast to HIV-1, we found that single missense mutations in the v1 loop of human TRIM5α were not able to restrict SIVsab, an SIV strain that infects sabers monkeys. Through combinatorial mutagenesis in both rhesus and human TRIM5α backbones, we show that human TRIM5α needs to acquire at least 6 rhesus-like changes in its v1 loop to gain restriction, including a 2 amino acid insertion, making it an insurmountable challenge. However, through a novel indel scanning method, we show that human TRIM5α can nevertheless overcome SIVsab via rare but highly effective small insertions in its v1 loop, opening the possibility of overcoming seemingly insurmountable viral challenges via indel instead of missense mutations.

Horizontal transmission of diverse transposable elements generates introns across the eukaryotic tree of life

Landen Gozashti[1] and Russell Corbett-Detig[*2]

[1] Department of Organismic and Evolutionary Biology – 26 Oxford Street Cambridge, MA 02138, United States
[2] University of California [Santa Cruz] – 1156 High Street, Santa Cruz, Ca 95064, United States

Abstract

Introns are a fundamental component of eukaryotic genome architecture with poorly understood origins. Recently, we proposed that intron generating transposable elements, introners, are the major driver of intron gain in diverse eukaryotic lineages. However, the molecular mechanism(s) and population processes of introner propagation remain elusive and the means of spread into new lineages is largely unknown. We analyze 8719 genomes, revealing 1695 novel intron generating TEs in 693 species spanning nearly every represented eukaryotic lineage. Introners contain functional protein domains and sequence features consistent with diverse TEs including canonical terminal-inverted-repeat DNA TEs, long terminal repeat retrotransposons and tyrosine recombinases as well as a myriad of novel TEs with uncharacterized molecular mechanisms. We identify several cases where introners have recently horizontally transferred between highly genetically divergent host species. Our results indicate that intron gain is an almost inevitable consequence of transposable element activity and transfer among eukaryotic lineages thereby resolving a central mystery of genome structure evolution.

Keywords: Intron, Splicing, Horizontal Gene Transfer, Genome Structure Evolution, Transcriptome

[*] Speaker

sciencesconf.org:icte2024:528660

Reverse Transcriptase-Related Genes at the Intersection of Environmental Stress Response Pathways

Irina Arkhipova*[1], Irina Yushenova[1], and Fernando Rodriguez[1]

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Abstract

In eukaryotes, domestication of reverse transcriptases (RT) is exceptionally rare. Throughout the course of evolution, it occurred during the major evolutionary transition converting circular into linear chromosomes in a primordial eukaryote, when telomerase RT (TERT) was recruited for maintenance of chromosome ends. This is in stark contrast to other retrotransposon ORFs (*gag*, *env*), which have been repeatedly co-opted for eukaryotic host functions. In another landmark evolutionary event, the RT domain was co-opted during the emergence of Prp8, the core component of the eukaryotic spliceosome, although it lost its polymerizing activity due to replacement of catalytic residues. The only known case of RT domain recruitment for catalytic function other than TERT is observed in *rvt* genes, a distinct type of RT which is widespread in free-living, mostly soil-dwelling, organisms, and is often subject to horizontal transfers. Most *rvt* genes are single-copy; harbor intact catalytic residues; and are exceptional in not being restricted to eukaryotes, raising the possibility that *rvt* domestication predated the emergence of TERT and Prp8. Here, we define the overlap in sets of host genes co-responding to different stresses accompanied by massive *rvt* induction in diverse hosts, revealing association with the corresponding host pathways; elucidate the molecular basis for conversion from templated to non-templated synthesis; uncover the capacity for self-organizing multimer formation upon horizontal transfers into heterologous hosts; and establish the nature of nucleic acids and extension products associated with rvt in its native host, enabling us to determine the prerequisites for efficient utilization of exogenous nucleic acids. Collectively, the data accumulated from phylogenetic, biochemical, genomic, transcriptomic, structural, and functional analyses of *rvt* genes in bacteria, fungi, and metazoans support their involvement in the host response to a diverse range of environmental stresses *via* template-independent polymerization, and outline the prerequisites for successful RT domestication.

Keywords: Reverse transcriptase, Domestication, Stress response, Adaptation

* Speaker

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Session II: TEs in health and disease

Escapee L1s promote parvalbumin interneuron development

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Abstract

Retrotransposons are mobile DNA sequences duplicated via transcription and reverse transcription of an RNA intermediate. Cis-regulatory elements encoded by retrotransposons can also promote transcription of adjacent genes. Somatic LINE-1 (L1) retrotransposon insertions have been detected in mammalian neurons. It is however unclear whether L1 sequences are mobile in only some neuronal lineages, or therein promote neurodevelopmental gene expression. Here we report programmed L1 activation by SOX6, a transcription factor critical for parvalbumin (PV) interneuron development. Mouse PV interneurons permit L1 mobilization in vitro and in vivo, harbor unmethylated L1 promoters, and express full-length L1 mRNAs and proteins. Using nanopore long-read sequencing, we identify unmethylated L1s proximal to PV interneuron genes, including a novel L1 promoter-driven Caps2 gene isoform that enhances neuron morphological complexity in vitro. These data highlight the contribution made by L1 cis-regulatory elements to PV interneuron development and transcriptome diversity, uncovered due to L1 mobility in this milieu.

Local heterochromatin mitigates the cis-regulatory impact of SVA transposons in human brain development and disease

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Abstract

SINE-VNTR-Alu (SVA) elements are hominoid-specific transposons that have entered our genomes during the last few million years of evolution. Strikingly, some SVAs are still active and able to retrotranspose, significantly contributing to polymorphisms in the human population. Such polymorphic SVA alleles harbour gene-regulatory potential and can lead to genetic disease. One notable example is X-Linked Dystonia-Parkinsonism (XDP), a neurodegenerative disorder caused by an SVA insertion at the *TAF1* locus. However, how these SVA insertions are controlled in the human brain and the function by which they impact human physiology and disease is unknown.

Here, we dissect the epigenetic regulation and influence of SVAs in various cellular models of X-Linked Dystonia-Parkinsonism. Employing a combination of CUT&RUN, Oxford Nanopore Sequencing and CRISPR approaches we demonstrate that the KRAB zinc finger protein ZNF91 orchestrates the establishment of H3K9me3 and DNA methylation over SVAs, including polymorphic ones, in a cell-type specific manner. The resulting miniheterochromatin domains not only silence SVA expression but also mitigate their *cis*-regulatory impact, thereby safeguarding the human genome from their effect. Interestingly, this regulatory mechanism proves to be crucial for XDP pathology. Removal of H3K9me3 and DNA methylation in patient derived neural progenitor cells severely aggravates the XDP molecular phenotype, resulting in increased *TAF1* intron retention and reduced expression.

Our results provide unique mechanistic insights into how human polymorphic transposon insertions are recognized, and their regulatory impact constrained by an innate epigenetic defence system. Furthermore, XDP serves as a suitable model to highlight the importance of this system within the context of disease.

Keywords: polymorphic insertions, SVA, DNA methylation, H3K9me3, XDP

* Speaker

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Assessment of L1 retrotransposition in DNA repair deficient transgenic mouse models

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Abstract

Long Interspersed Element 1 (L1) retrotransposons are endogenous mutagens that cause *de novo* inserts and DNA breaks. L1 overexpression can trigger apoptosis, senescence, large genomic deletions, and inflammatory response in cultured cells. Many of these events result from involvement of various cellular DNA repair pathways. Several DNA repair genes have been reported to suppress L1 retrotransposition in various experimental systems. However, how these genes and their defects affect L1 retrotransposition in normal mammalian environment remains poorly understood. We have developed a custom transgenic mouse model, harboring a single copy of a human L1 transgene to assess its mobilization *in vivo*. The L1Tg generates *de novo* inserts containing typical marks of authentic L1 integration. L1Tg-specific ddPCR analysis of DNA from large cohorts of wild type mice shows that the rate of L1Tg mobilization varies between genetic backgrounds. Breeding of these L1 transgenic mice with Xpc-deficient mice shows that a complete loss of Xpc increases *de novo* L1Tg retrotransposition. In contrast, Tp53-deficiency results in the decrease in *de novo* L1Tg mobilization in heterozygous and homozygous mice compared to the wild type mice. Changes in the L1 transgene retrotransposition in the germline or early embryo likely account for the observed differences in both Xpc- and Tp53-deficient mice. Our L1 transgenic mouse model and our findings open opportunities to investigate the health impact of L1 retrotransposition *in vivo* in the context of various genetic deficiencies and environmental exposures relevant to human diseases or their treatment. Our findings also reflect the complexity of L1 host interactions in mammalian systems that can be further exploited to understand mechanisms underlying heterogeneity in *de novo* L1 mobilization observed in the human population.

Keywords: DNA repair, retrotransposition, mouse models

* Speaker

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The aging repeatome: connection to chronic inflammation

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Abstract

The genomes of virtually all organisms contain repetitive sequences that are generated by the activity of transposable elements (transposons). This relationship has been largely competitive, and transposons have earned epithets such as *'junk DNA'* and *'molecular parasites'*. All organisms have evolved diverse mechanisms to repress the activity of their transposons. Recent evidence suggests that retrotransposable elements (retrotranposons) can become activated with age in somatic tissues of diverse species including yeast, *Drosophila*, and mammals. In particular, during cellular senescence of human cells, LINE-1 retrotransposons become transcriptionally derepressed and activate a type-I interferon (IFN-I) response. The IFN-I response appears to be triggered by L1 cDNAs synthesized by the L1 reverse transcriptase. LINE-1 derepression occurs in late senescence and contributes to the maintenance of a canonical pro-inflammatory property of senescent cells known as the *'senescence-associated secretory phenotype'* (SASP). At the organismal level the SASP has been implicated in promoting age-associated sterile inflammation in diverse tissues (now often referred to as *'inflammaging'*). Somatic activation of retrotransposons might be a hitherto unappreciated driver of inflammaging, and some of the consequent pathologies might be ameliorated therapeutically by inhibiting reverse transcriptase, or other functions encoded by retrotransposons.

Role of retrotransposons in insect antiviral immunity

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Abstract

Mosquitoes show a remarkable ability to sustain persistent viral infections without obvious signs of disease. Known as disease tolerance, this property is critical to the transmission of major human pathogens, such as dengue, Zika and yellow fever viruses. We previously discovered that upon RNA virus infection of Drosophila melanogaster, fly survival requires the synthesis of viral DNA species by retrotransposon-encoded reverse transcriptases (RTs), which boost antiviral defenses. Interestingly, treating Aedes mosquitoes with RT inhibitors also resulted in the death of infected insects, leading us to hypothesize that retrotransposon-virus interactions enable mosquitoes to tolerate infection and transmit viruses. However, the identity and function of the retrotransposons involved, and the relevance to viral tolerance in natural mosquito populations remain unknown. Here, I will present our current efforts towards elucidating how retrotransposons shape the equilibrium between insects and viruses and their physiological roles in immunity.

Endogenous Retrovirus-derived RNAs play an essential role during morphogenesis in zebrafish

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Abstract

ERVs have shaped the genomes of vertebrates, however, the extent of their influence on evolution, functionality, and disease remains to be elucidated. Although most ERVs have lost the ability to mobilize, their transcription is tightly regulated from pluripotency to morphogenesis, suggesting a crucial role for these elements. During early embryogenesis, various signaling pathways control gene regulatory networks and play a pivotal role in body plan formation. Using zebrafish as a model, we wondered how these signaling pathways impact ERVs expression during morphogenesis. We analyzed expression data from embryos with perturbed retinoic acid, Wnt, FGF and Nodal signaling pathways during early embryogenesis. We observed that the expression of several ERVs was affected, particularly the ERV1-3 subfamily was upregulated by retinoic acid overexpression and downregulated by Nodal signaling inhibition. These results together with the specific expression pattern suggest that ERV1-3 transcripts might play a role during axis specification. Using CRISPR-RfxCas13d approaches, we demonstrate that several guides targeting different regions of the internal part and the LTRs of ERV1-3 elements reduced its transcription levels and replicated a robust and specific phenotype related to Nodal signaling inhibition. To gain mechanistic insights into which specific ERV-derived RNAs are responsible for this phenotype, we conducted genomic and functional characterizations of this subfamily in addition to a differential expression analysis at locus-specific level. We found that only three full-length ERV1-3 copies exhibited significant downregulation and all of these copies show an enrichment of adaxial and paraxial mesoderm transcription factors binding motifs in their LTRs. Additionally, these copies were characterized by the presence of protease, reverse transcriptase, RNAse-H, and integrase ORFs with varying degrees of completeness. Finally, using one of these copies, we partially rescued the phenotype. Altogether, this study provides insights into the cooption of ERV mRNAs during development and their ability to generate new developmental functions.

Keywords: Endogenous retrovirus, zebrafish, development

[*] Speaker

A retrotransposon in cancer : the Marker and the Mutator

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Abstract

The human genome is replete with repetitive DNA attributable to the activity of self-propagating genetic sequences. In humans, this landscape is dominated by retrotransposons that make new copies of themselves by first being transcribed to RNA intermediates and then reverse transcribed to cDNA that is ultimately integrated into the genome. Long interspersed element-1 (LINE-1)-encoded proteins are responsible for this process, which occurs both in the germline and in somatic tissues. Increased expression and animation of genomic LINE-1 sequences appear to be hallmarks of cancer, and can be responsible for driving mutations in tumorigenesis. LINE-1 sequences encode a 6-kilobase (kb), bicistronic RNA intermediate transcribed from an internal RNA polymerase II promoter. The first of its open reading frames (ORFs) encodes ORF1 protein (ORF1p), which forms an RNA-binding homotrimer. Here, I will review data that this protein can serve as a marker of malignancies both in tissue biopsies and in the peripheral blood of cancer patients. The second ORF encodes ORF2p, which encompasses endonuclease and reverse transcriptase domains. These act in a coordinated manner to generate *de novo* genomic LINE-1 insertions, mutating cancer genomes. Here, I will review published and preliminary data that L1-mediated mutagenesis generates frequent double stranded (ds)DNA breaks leading to chromosomal deletions and inciting structural chromosomal instability (CIN). These findings suggest that LINE-1 may commonly contribute to cancer development and that enhancing its DNA damaging effects may represent a therapeutic strategy.

Intercellular propagation of neurodegeneration by endogenous retroviruses

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Abstract

Inter-cellular movement of "prion-like" proteins is thought to explain propagation of neurodegeneration between cells. For example, propagation of abnormally phosphorylated cytoplasmic inclusions of TAR-DNA-Binding protein (TDP-43), followed by a prion-like templating mechanism, is proposed to underlie progression and inter-cellular spread of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). But unlike transmissible prion diseases, ALS and FTD are not infectious and injection of aggregated TDP-43 is not sufficient to cause disease as is the case with PrP. Such injections of TDP-43 seeds does result in intercellular spread of the TDP-43, leading to appearance of pathological changes in the protein in neurons distant from the injection site, but the disease does not progress and the recipient neurons have not been found to undergo toxic decline. This suggests a missing component of a positive feedback necessary to sustain disease progression. We demonstrate that endogenous retrovirus (ERV) expression and TDP-43 proteinopathy are mutually reinforcing. Expression of either *Drosophila* mdg4-ERV (gypsy) or the human ERV, HERV-K (HML-2) are each sufficient to stimulate cytoplasmic aggregation of human TDP-43. ERV expression stimulates such aggregation both in cell culture and in the *Drosophila* brain. In flies, glial TDP-43 pathology can drive both pathological protein accumulation and cell death in nearby glia and neurons, and this intercellular effect requires mdg4 in the glial cells. Viral ERV transmission between cells grown in culture also can trigger TDP-43 pathology in recipient cells, whether they are grown in contact or at a distance. This suggests a new mechanism by which neurodegenerative effects may propagate through neuronal tissue. Structure-function manipulations of mdg4 and of HERV-K point to mechanistic underpinnings by which ERVs may trigger TDP-43 proteinopathy.

Nuclear translocation of LINE-1 encoded ORF1p alters nuclear envelope integrity in human neurons

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Abstract

LINE-1 retrotransposons are emerging as possible culprits in neurodegenerative diseases and provide novel targets for disease-modifying therapies. However, the molecular mechanisms underlying the pathogenic role of LINE-1 and their encoded proteins ORF1p and ORF2p are still not completely understood. While the endonuclease and reverse transcriptase activity of ORF2p has been associated with DNA damage and inflammation, no pathogenic role has yet been assigned to ORF1p. To address this question, we established an in vitro human neuronal model displaying a robust increase of LINE-1 activity and ORF1p upon application of arsenite. ORF1p increase was accompanied by a loss of nuclear envelope integrity, disruption of nucleo-cytoplasmic transport including the cytoplasmic mislocalization of TDP-43 and heterochromatin destructuration, which are established or emerging features of aging and/or associated with neurodegenerative diseases. Arsenite-favored translocation of ORF1p into the nucleus was mediated by interaction of ORF1p with nuclear import receptors, nuclear pore complex components and nuclear lamina proteins. Blocking ORF1p nuclear import or stabilizing the nuclear envelope with the small molecule remodelin normalized nuclear ORF1p levels and restored nuclear envelope integrity, nucleo-cytoplasmic transport and heterochromatin organization. Overexpression of ORF1p in the absence of arsenite recapitulated nuclear envelope dysfunctions and loss of nuclear circularity correlated with nuclear ORF1p levels. This study thus reveals a retrotransposition-independent pathogenic action of ORF1p perturbing nuclear envelope integrity.

Keywords: Retrotransposons, LINE, 1, ORF1p, nuclear envelope, nucleo, cytoplasmic transport, neurodegeneration, aging, chromatin, genomic instability

* Speaker

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Session III: TE control and epigenetics

Keynote lecture

Programmed genome elimination in Paramecium, a definitive form of transposon silencing

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Abstract

Our team studies the remarkable process of small-RNA-guided DNA elimination that occurs during development in the eukaryote Paramecium, in which at least a third of the genome is reproducibly removed. Our efforts aim to understand the mechanisms that control these events, as well as the evolutionary trajectories of the eliminated sequences. Our approach combines molecular and cellular genetics, biochemistry and large-scale genomic strategies.

A dual histone code specifies the binding of heterochromatin protein Rhino to a subset of piRNA source loci

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Animal germ cells deploy a specialized small RNA-based silencing system, called the PIWI-interacting RNA (piRNA) pathway, to prevent aberrant expression of transposable elements and maintain genome integrity. In *Drosophila* germ cells, the majority of piRNA populations originate from dual-strand piRNA clusters, genomic regions highly enriched in transposon fragments, via an elaborate protein machinery centred on the heterochromatin protein 1 homolog, Rhino. Although Rhino binds to peptides carrying trimethylated H3K9 in vitro, it is not fully understood why it only occupies a fraction of H3K9me3-decorated heterochromatin in vivo. Recent work uncovered that Rhino is recruited to subsets of piRNA source loci by the zinc finger protein Kipferl. Here we identify a Kipferl-independent mode of Rhino targeting that is dependent on the histone H3 lysine 27 methyltransferase Enhancer of Zeste and the presence of H3K9me3 and H3K27me3 marks. Using a Kipferl-independent system, we find that Rhino, through a chromodomain dimer, specifically binds to loci marked by both H3K9me3 and H3K27me3. These results expand our understanding of the characteristic binding profile of the heterochromatin protein Rhino and reveal a role for dual histone modifications in defining the specificity of a chromatin binding protein.

Keywords:
Epigenetics, *Drosophila*, piRNA pathway, transposon silencing, Heterochromatin

SUMOylation ”hot spots” in piRNA-mediated TE silencing pathways revealed by diGly proteomics

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Abstract

The piRNA pathway is central to the transcriptional and post-transcriptional control of transposon activity within animal germ cells. Despite notable advancements, a comprehensive understanding of the molecular mechanisms underlying piRNA biogenesis and function remains elusive. Our prior work demonstrated that depletion of the small ubiquitin-like modifier (SUMO) from the Drosophila female germline leads to substantial gene deregulation, aberrant transposon activation, and infertility. Nevertheless, the specific targets and mechanistic roles of SUMOylation within diverse biological contexts, including in the piRNA pathway, have been hindered by technical challenges. To investigate the role of protein modification by SUMO in the Drosophila ovary further, we devised a novel transgenic model and compatible diGly proteomics-based strategy for unbiased discovery of SUMO targets with aminoacid-level precision. This approach revealed a comprehensive set of SUMO targets, prominently enriched in proteins connected to the piRNA pathway and heterochromatin (Ninova et al., 2023, *Cell Genomics* 3, 100329). Intriguingly, while SUMO is known to primarily affect nuclear proteins, we found multiple SUMO modifications on proteins localized within the germ granule-like compartment ’nuage” - the site of piRNA biogenesis and posttranscriptional transposon cleavage. Furthermore, we found that multiple piRNA pathway proteins undergo regulated multi-SUMOylation, dependent on the central epigenetic silencing effector Piwi. These results collectively indicate a wide-ranging and multifaceted role of protein SUMOylation in chromatin regulation and transposon control extending beyond previously recognized functions, thereby opening new avenues in the field.

Keywords: transposons, piRNAs, Piwi, drosophila, heterochromatin, SUMO

* Speaker

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Retrotransposon addiction promotes centromere function via epigenetically activated small RNAs

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Abstract

Retrotransposons have invaded eukaryotic centromeres in cycles of repeat expansion and purging, but the function of centromeric retrotransposons, if any, has remained unclear. In *Arabidopsis*, centromeric *ATHILA* retrotransposons give rise to epigenetically activated short interfering RNAs (easiRNAs) in mutants in *DECREASE IN DNA METHYLATION1 (DDM1)*, which promote histone H3 lysine-9 di-methylation (H3K9me2). Here, we show that mutants which lose both DDM1 and RNA dependent RNA polymerase (RdRP) have pleiotropic developmental defects and mis-segregation of chromosome 5 during mitosis. Fertility defects are epigenetically inherited with the centromeric region of chromosome 5, and can be rescued by directing artificial small RNAs to a single family of *ATHILA5* retrotransposons specifically embedded within this centromeric region. easiRNAs promote pericentromeric condensation, chromosome cohesion and proper chromosome segregation in mitosis. We propose that insertion of *ATHILA* silences centromeric transcription, while simultaneously making centromere function dependent on retrotransposon small RNAs in the absence of DDM1, potentially promoting the selfish survival and spread of centromeric retrotransposons. Parallels are made with the fission yeast *S. pombe*, where chromosome segregation depends on RNAi, and with humans, where chromosome segregation depends on both RNAi and HELLS^{DDM1}.

Transposable elements and antiviral immunity

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Abstract

Abstract: Transposable elements (TEs) are parasite DNA sequences that are controlled by RNA interference pathways in many organisms. In insects, antiviral immunity also is achieved by the action of small RNAs. We used Drosophila and different viruses to analyze the reciprocal impacts of viral infections on TEs. We found that the antiviral response modulated TE transcript amounts, with the clearest effects in somatic tissues. In addition, using Drosophila C Virus, we found that TEs are involved in a dual response: on the one hand TE control is released upon DCV infection, and on the other hand TE transcripts are associated with a reduction of viral replication. This discovery highlights a pivotal role for TEs in the long-term arms race between a virus and its host.

Keywords: virus, RNA interference, small RNA, transcriptomics

[*] Speaker

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The piRNA pathway mediates transcriptional silencing of LTR-retrotransposons in the soma and germline of Aedes mosquitoes

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Abstract

The PIWI-interacting RNA (piRNA) pathway is crucial for maintaining genomic integrity by suppressing transposable elements in animal germlines. Despite this recognized germline function, piRNAs are widely expressed in somatic tissues across arthropod species, raising questions about somatic functions of the piRNA pathway. Here, we studied the *Aedes albopictus* mosquito, which expresses four out of its seven PIWI genes in both germline and somatic tissues. We generated *Piwi6* knockout *Ae. albopictus* cell lines, and observed a substantial upregulation of Long Terminal Repeat (LTR)-retrotransposons. Prominent among these is an element that seems to encode intact *gap*, *pol* and *env* genes, which we named Aedes endogenous retrovirus 1 (AeERV1). Our findings indicate that Piwi6 transcriptionally silences AeERV1 by depositing repressive H3K9me3 histone marks, leading to heterochromatin formation. Intriguingly, *Piwi6* gene knockdown in adult *Ae. albopictus* mosquitoes resulted in AeERV1 upregulation in both somatic and germline tissues. Our data indicate that piRNAs mediate transcriptional transposon silencing in the mosquito soma and suggest that this function may be evolutionarily conserved across arthropod species.

Keywords: PIWI protein, piRNA pathway, LTR retrotransposon, endogenous retrovirus, epigenetics, transcriptional silencing

[*] Speaker

sciencesconf.org:icte2024:525238

A new spin to chromatin regulation of retrotransposons

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Abstract

Multilayered mechanisms safeguard host genomes against the activity of transposable elements, hitting them at different stages of their life cycle. Deployment of countermeasures is particularly crucial in germ cells and their precursors in the early embryo. This is where the relative interests of the host versus transposable elements are the most conflicting, and from their outcome depend reproductive success and species fitness. We will present our latest efforts in uncovering the diversity of repressive pathways that concur towards keeping transposable elements quiet within the mammalian reproduction window.

DNA methylation enables recurrent endogenization of giant viruses in a protist closely related to animals

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Abstract

5-methylcytosine (5mC) is a widespread silencing mechanism that controls genomic parasites. However, in many eukaryotes 5mC has gained complex roles in gene regulation beyond parasite control. Animals are a quintessential case for 5mC evolution, as they show widespread variability across lineages, ranging from gene regulation and transposable element control to loss of this base modification. Here we show that the protist closely related to animals, Amoebidium appalachense, features both transposon and gene body methylation, a pattern reminiscent of invertebrates and plants. Unexpectedly, large hypermethylated regions of the Amoebidium genome derive from viral insertions, including hundreds of endogenized giant viruses contributing 14% of the encoded genes, to an extent never reported before in any eukaryotic genome. Using a combination of inhibitors and functional genomic assays, we demonstrate that 5mC silences these giant virus insertions. Moreover, alternative Amoebidium isolates show polymorphic giant virus insertions, highlighting a dynamic process of infection, endogenization and purging. Our results indicate that 5mC is critical for the controlled co-existence of newly acquired viral DNA into eukaryotic genomes, making Amoebidium a unique model to understand the hybrid origins of eukaryotic genomes.

Keywords: Giant virus, DNA methylation, Epigenetics, Endogenisation, Evolution, Genome growth

* Speaker

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Locus-level L1 DNA methylation profiling reveals the epigenetic and transcriptional interplay between L1s and their integration sites.

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2 Epigenetics and Cell Fate – University Paris Cité, CNRS UMR 7216 – 35 rue Hélène Brion 75205 Paris Cedex 13, France

Abstract

LINE-1 (L1) retrotransposons represent an abundant and repetitive fraction of the human genome and are implicated in human disease and evolution. Epigenetic mechanisms, such as DNA methylation, suppress their activity in most somatic tissues, but they are transcribed and eventually mobilized in many cancers. Consistently, loss of bulk L1 DNA methylation is a common cancer biomarker, and demethylating agents used in cancer therapy further increase L1 expression, contributing to the establishment of a viral mimicry state. However, given the repetitive nature and the dispersion of L1 sequences throughout the genome, deciphering the regulation of individual copies has been challenging. Here, we combine short- and long-read sequencing to unveil L1 methylation variation across celltypes, families and individual loci and elucidate key principles involved. We find that the youngest primate L1 families are specifically hypomethylated in pluripotent stem cells and the placenta, but not in most tumors. Locally, intronic L1 methylation is intimately associated with gene transcription. Conversely, the L1 methylation state can propagate to the proximal region up to 300 bp. This phenomenon is accompanied by the binding of specific transcription factors, which drive the expression of L1 and chimeric transcripts. Finally, L1 hypomethylation alone is typically insufficient to trigger L1 expression due to redundant silencing pathways. Together, our results highlight the reciprocal influence of L1 retrotransposons and their integration sites with respect to DNA methylation and expression, and reveal unanticipated layers of cell-type-specific epigenetic regulation. This work was supported by Agence Nationale de la Recherche (ANR-11-LABX-0028; ANR-11-LABX-0071; ANR-15-IDEX-0001; ANR-16-CE12-0020; ANR-18-IDEX-0001; ANR-19-CE12-0032; ANR21-CE12-0001), Fondation pour la Recherche M´edicale (DEQ20180339170), Institut National Du Cancer (INCa PLBIO 2020-095), Fondation ARC (PGA1/RF20180206807), and other grants from the Cancerop^ole PACA, INCa and the Region Sud (Projet Emergence), INSERM (GOLD Cross-cutting Program on Genomic Variability), and CNRS (GDR 3546).

Keywords: L1, DNA methylation, nanopore sequencing, methylation array, L1 chimeric transcripts, transcription factor, genomic profiling

* Speaker

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Fighting back epigenetic control: A story about the evolution and function of transposon-encoded anti-silencing systems

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Abstract

Epigenetic control of transposable elements (TEs) imposes strong selective constraints, engaging hosts and TEs in contested evolutionary arms races. An expected outcome of these arms races is the emergence of TE-encoded counteracting systems. However, the existence of such counteracting responses remains obscure. Here, we present the discovery and characterization of evolutionary unrelated anti-silencing systems encoded by distinct DNA transposons in plants. A shared characteristic of these independent systems is their remarkable sequence specificity, which relies on TE-encoded regulatory factors harboring DNA-binding domains of unknown origin, along with multimerization domains. These factors have the ability to bind target DNA sequences embedded in highly heterochromatic sequences, akin to bona fide pioneer transcription factors, and induce specific loss of DNA methylation and epigenetic activation of targeted TEs. Last, we also demonstrate that the activity of TE-encoded anti-silencing factors could account for the hitherto enigmatic Enhancer/Suppressor function described by Barbara McClintock more than 60 years ago. Our findings reveal how conflicts between TEs and hosts have driven the evolution of controlling systems.

Keywords: epigenetics, anti, silencing, arms race, plants, DNA transposons, DNA methylation

* Speaker

Session IV: Transposition mechanisms and applications

Molecular innovation at the CRISPR-transposon interface

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Abstract

CRISPR-Cas systems protect bacteria and archaea from foreign invaders, including viruses and plasmids. Paradoxically, though, CRISPR-Cas evolution involved the repeated co-option of genes from another major class of mobile genetic elements: transposons. In turn, CRISPR-Cas systems have been co-opted by transposons to promote a new mode of mobilization involving RNA-guided DNA insertion. Thus, dynamic co-evolution at the host-transposon interface has had a profound influence on the diversification of prokaryotic adaptive immune systems and the functionalization of RNA-guided targeting enzymes. I will present our efforts to investigate the molecular functions of RNA-guided nucleases and nuclease-deficient CRISPR-Cas systems in transposition, focusing on CRISPR-associated transposases (CASTs) and TnpB-family enzymes. Recent experiments revealed the unexpected emergence of RNA-guided transcription factors through recurrent TnpB gene domestication events, leading to diverse modes of programmable gene regulation.

DNA folding and sequence asymmetry promote conjugative transposition of antibiotic resistance

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Abstract

Conjugative transposons drive horizontal gene transfer and promote the spread of antibiotic resistance genes across bacteria. Their movements have been linked to the emergence of multidrug-resistant pathogens, which present a critical health challenge worldwide. Yet, their molecular mechanisms are unclear.

Using a dedicated computational pipeline, we surveyed the most wide-spread conjugative transposons and characterized their diversity, genetic cargo, and transfer dynamics. Focusing on a broadly distributed element from Gram-negative pathogens, we dissected the biochemical pathway and determined high-resolution crystal and cryo-EM structures of multicomponent protein-DNA assemblies involved in its movement. The structures capture various steps of transposition and reveal an intricate interplay between the core transposition machinery and host-encoded proteins. A community of protein molecules folds transposon and genomic DNA in unique ways to cut, exchange and re-join DNA strands in a tightly regulated manner. Asymmetric DNA recognition, assembly and cleavage mechanisms facilitate transposition irrespective of the genomic insertion site, expanding gene transfer across species.

These results shed new light onto the molecular strategies of conjugative transposons and, combined with bioinformatic data, show how they evolved to effectively spread genetic traits, such as antibiotic resistance.

A developmental condensin I complex assists the *Paramecium* PiggyMac domesticated transposase to carry out programmed DNA elimination

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Abstract

With its nuclear dimorphism, the ciliate *Paramecium tetraurelia* provides an original model to study the dynamics of transposable elements (TEs) within their host genome. The germline genome, hosted in the transcriptionally silent micronuclei (MIC), has been colonized by TEs, including in coding regions. At each sexual generation, TEs and thousands of related sequences are removed from the somatic genome, when the macronucleus (MAC), dedicated to gene expression, develops from a copy of the MIC. Several components of the programmed DNA elimination (PDE) machinery are known, including the PiggyMac (Pgm) endonuclease and its five Pgm-like partners (PgmL1 to PgmL5). However, how the DNA cleavage activity of Pgm is controlled during PDE has remained elusive.

In this study, a TurboID screen uncovered the presence of two condensin I subunits, Smc2 and CapD2.3, in the proximal proteomes of Pgm and PgmL4. Condensin is a conserved pentameric complex known for its role in chromosome compaction and segregation during mitosis and meiosis. A combination of transcriptome analyses and tandem immunoprecipitation experiments from *Paramecium* nuclear extracts allowed us to identify a developmental condensin I complex, formed by the association of three development-specific subunits, Smc4.2, CapD2.3 and CapH.3, with two constitutive subunits, Smc2 and CapG. We report that Smc4.2 and CapD2.3 localize exclusively in the developing MACs, and that *SMC4.2*, *CAPD2.3* or *CAPH.3* knockdowns block PDE, leading to death of sexual progeny. Moreover, depleting cells of any of the three developmental subunits destabilizes the localization of Pgm and its partners within the developing MAC, by the time PDE takes place. CapD2.3 immunoprecipitation experiments further showed a physical interaction between the condensin I and the endonuclease complexes. Our results indicate that the developmental condensin complex assists the Pgm endonuclease during PDE in *P. tetraurelia*, highlighting a non-canonical role of a eukaryotic condensin in a non-mitotic process.

Redefining mobility in bacterial genetics

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Abstract

Bacterial infections are a leading cause of global mortality, and the emergence of new multi-drug resistant clones continues to be a concern. Therefore, it is crucial for us to comprehend the processes that contribute to the evolution of virulence and antibiotic resistance. Traditionally, it has been believed that mobile genetic elements (MGEs) play significant roles in these processes due to their ability to horizontally spread within bacterial populations. In contrast, bacterial chromosomes have been traditionally considered mostly immobile within the cell, serving as a framework for generating diverse genomes through the horizontal acquisition of exchangeable genes. However, the recent discoveries of phage- and PICI-mediated lateral transduction challenge this model. We have recently demonstrated that lateral transduction can facilitate the mobility of core genes in bacterial chromosomes at frequencies higher than those observed for elements traditionally regarded as mobile. This raises important questions regarding the definition of an MGE and the impact of this phenomenon on bacterial populations. In this presentation, we aim to generalize the concept that the mobility of bacterial chromosomes surpasses that of many MGEs. Furthermore, we will discuss the relevance of these findings in driving the emergence of novel virulent and multi-resistant bacteria, as well as preserving the integrity of successful pathogenic clones.

Integron cassettes commonly integrate into bacterial genomes via widespread non-classical attG sites

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8 Viral DNA Integration and Chromatin Dynamics Network (DyNAVir) – Université de Bordeaux (Bordeaux, France) – Bordeaux, France

Abstract

Integrons are genetic elements involved in bacterial adaptation. They contain an integrase and an array of gene cassettes whose expression decreases with distance from the beginning of the array. Stress activates the integrase leading to capture and shuffling of cassettes through site-specific recombination between *attC* and *attI* integron sites. The concomitant changes in cassette expression allow the exploration of diverse phenotypic combinations. Here we demonstrate that the integrase also catalyzes cassette integration into bacterial genomes outside of the known *att* integron sites. Once integrated, these cassettes can be expressed if located near bacterial promoters and can be excised at the integration point or outside, inducing chromosomal modifications in the latter case. Analysis of more than 5 × 105 independent integration events revealed a very large genomic integration landscape. We identified consensus recombination sequences, named *attG* sites, which differ greatly in sequence and structure from classical *att* sites. These results unveil an alternative route for dissemination of adaptive functions in bacteria and expand the role of integrons in bacterial evolution.

Keywords: Bacterial evolution, site, specific recombination, integron

* Speaker

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Plant genome stability and extrachromosomal circular DNA (eccDNA): vicious or virtuous circle?

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Abstract

The presence of extrachromosomal circular DNA (eccDNA) is associated with transposable element (TE) activity. However, both the epigenetic regulation of eccDNA and its impact on genome stability are poorly studied. I will illustrate how the study of eccDNA can illuminate genome dynamics. In Arabidopsis, using long reads, we sequenced the eccDNA compartment and the genome of *Arabidopsis thaliana* mutant plants affected in DNA methylation and post-transcriptional gene silencing. In these mutants, a high load of TE-derived eccDNAs with truncated and chimeric forms was associated with truncated and full-length TE neo-insertions in the genome. Additionally, we detected complex structural variations (SVs) notably at a disease resistance cluster, and several large tandem duplications specific of each mutant individual, suggesting that SVs have been overlooked in epigenetic mutants. We propose that a high load of eccDNA may alter DNA repair pathways triggering the accumulation of SVs. These observations could have implications in cancer cells where eccDNAs are abundant.

Keywords: eccDNA, LTR retrotransposon, DNA methylation, RdDM, mobilome, seq

* Speaker

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Temporal and spatial partitioning of retrotransposon niches in *Drosophila melanogaster*.

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Abstract

Transposable elements (TE) abundance within the genome suggests that they have certainly adapt their modes of expression/integration upon genome colonization, to insure their proper maintenance and propagation. To explore further these mechanisms, we constructed a Drosophila melanogaster line in which the mobility of different TE families can be induced due to the ovarian somatic relief of the piRNA pathway (Barckmann et al., 2018). Thanks to long-read DNA sequencing and a bio-informatic pipeline established in the laboratory (TrEMOLO, https://github.com/DrosophilaGenomeEvolution/TrEMOLO) (Mohamed et al., 2023), we precisely determined novel integration sites for four TE families belonging to the endogenous retrovirus group. Interestingly, two of them (ZAM and gtwin) exhibit a dynamic choice of their landing sites. Indeed, we showed that these TEs, to limit competition, have specific expression patterns and integration sites within the host genome. Additionally, we established that their timing of integration during embryogenesis varies.

Structures, Functions, and Adaptations of the Human LINE-1 ORF2 Protein

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Abstract

The LINE-1 (L1) retrotransposon is an ancient genetic parasite that has written around one third of the human genome through a ”copy-and-paste” mechanism catalyzed by its multifunctional enzyme, open reading frame 2 protein (ORF2p). ORF2p reverse transcriptase (RT) and endonuclease activities have been implicated in the pathophysiology of cancer, autoimmunity, and aging, making ORF2p a potential therapeutic target. However, a lack of structural and mechanistic knowledge has hampered efforts to rationally exploit it. We report structures of the human ORF2p ‘core’ (residues 238-1061, including the RT domain) by X-ray crystallography and cryo-EM in multiple conformational states. Our analyses reveal two novel folded domains, extensive contacts to RNA templates, and associated adaptations that contribute to unique aspects of the L1 replication cycle. Computed integrative structural models of full-length ORF2p show a dynamic closed ring conformation that appears to open during retrotransposition. We characterize ORF2p RT inhibition and reveal its underlying structural basis. Imaging and biochemistry reveal that non-canonical cytosolic ORF2p RT activity can produce RNA:DNA hybrids, activating innate immune signaling via cGAS/STING and resulting in interferon production. In contrast to retroviral RTs, L1 RT is efficiently primed by short RNAs and hairpins, which likely explains cytosolic priming. Additional biochemical activities including processivity, DNA-directed polymerization, nontemplated base addition, and template switching together allow us to propose an updated L1 insertion model. Finally, our evolutionary analysis reveals structural conservation between ORF2p and other RNA- and DNA-dependent polymerases. We therefore provide key mechanistic insights into L1 polymerization and insertion, shed light on L1 evolutionary history, and enable rational drug development targeting L1. (if time permits and the committee finds this of interest, unpubilshed structural and mechanistic data will also be presented)

Keywords: LINE, 1, ORF2p, Structure, Priming, Insertion mechanisms, Enzymology, Innate immune activation, interferon, cGAS, STING

[*] Speaker

Genetic Architects: How the LINE-1 Transposon Crafts a Third of Your Genome

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Abstract

Retrotransposons are mobile genetic elements in the human genome that are recognized as drivers of genome expansion and evolution. The Long Interspersed Element-1 (LINE-1) retrotransposon has generated over one-third of the human genome and serves as an active source of genetic diversity and human disease. Yet, how LINE-1 mobilizes within the human genome remains poorly understood. I will discuss our efforts to biochemically reconstitute the mobility mechanism by the human LINE-1 encoded enzyme with purified components. Our reconstitutions demonstrate how the LINE-1 enzyme nicks the target DNA to prime reverse transcription of the LINE-1 or SINE RNAs in vitro.

Using cryo-electron microscopy, we have obtained structures of retrotransposition intermediates with the LINE-1 enzyme engaging its native RNAs, e.g. the SINE RNAs, and target DNA to prime reverse transcription. We visualize extensive interactions with the singlestranded RNA and RNA secondary structures by five distinct domains of the LINE-1 enzyme, including sequence-specific contacts. Most surprisingly, we demonstrate an unexpected target-site requirement for DNA cleavage and reverse transcription where the enzyme recognizes an upstream single-stranded DNA to position adjacent DNA duplex in the endonuclease active site for nicking, generating a staggered DNA break with a single nick. These findings demonstrate that LINE-1's mobility is coupled to the DNA replication within human cells. In summary, our work provides key insights into the mechanism of ongoing transposition in the human genome and informs the engineering of retrotransposon proteins for gene therapy.

Keywords: LINE1, retrotransposon, cryoelectron microscopy, RNA, retroelement, retrotransposition

* Speaker

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Abstracts

Poster presentations in alphabetical order *per* topic (including Flash talks)

TEs, genome evolution and adaptation

Recurrent molecular adaptation revealed by systematic cross-species protein interaction analyses of the Drosophila piRNA pathway

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The piRNA pathway suppresses transposable elements in germline cells across the animal kingdom. However, despite their essential function for fertility, piRNA pathway genes of animals with diverse active transposon families, such as fruit flies and teleost fish, are known to be rapidly evolving. The functional impact of such rapid evolution of essential genome defence genes remains enigmatic. To address this question, we performed a yeast-two-hybrid screen to systematically test protein-protein interactions of eleven orthologous genes involved in piRNA precursor expression from five *Drosophila* species. Our data identify several conserved protein-protein interactions not impacted by rapid sequence evolution, but also reveal two types of molecular innovation within the *Drosophila* piRNA pathway: (1) co-evolution of PPIs as shown by species incompatibilities in protein-protein interactions that are otherwise conserved between orthologs from the same species, and (2) protein interaction rewiring exemplified by the species-specific recruiters of CtBP, a co-factor required to suppress canonical transcription of transposons at Rhino-occupied loci. Combined with evolutionary analyses and complementary protein-protein interactions assays our data uncover how an arms race, such as the one between transposons and the piRNA pathway in *Drosophila*, can lead to recurrent innovation of conserved protein interaction networks while preserving the pathway's core function.

Keywords: piRNA pathway, evolution, protein, protein interactions, transposon silencing

*Speaker

When lianas and trees talk DNA: new insights into the genetic exchanges in plants

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Horizontal transfer (HT) refers to the exchange of genetic material between divergent species, without reproduction. HT has played a significant role in bacterial evolution, but it is underestimated in higher eukaryotes. Several recent studies have demonstrated HTs in eukaryotes, particularly in the context of parasitic relationships and model species. However, very little is known about the HT in natural ecosystems, particularly those involving non-parasitic wild species and the nature of relationships that promote these HTs. To fill this knowledge, we conducted a pilot study to investigate HTs in a natural ecosystem, the Massane forest located in south of France by sequencing the genomes of 17 wild non-model species. To reach this goal, we developed a new computational pipeline called INTERCHANGE, allowing the characterization of HTs at the whole genome level without prior annotation and directly in the raw sequencing reads. Using this pipeline, we identified 12 HT events, half of which occurred between lianas and trees. We found mainly low copy number of LTR-retrotransposons from Copia superfamily are transferred between wild plant species, especially those of the Ivana and Ale lineages. This work highlights a new possible route for HTs between non-parasitic plants and provide new insights into genomic characteristics of horizontally transferred DNA in plant genomes.

Keywords: Horizontal transfers, LTR retrotransposons, Ecosystem, Non assembled genomes, Comparative genomics

*Speaker

Recent reactivation of a pathogenicity-associated transposable element is associated with major chromosomal rearrangements in a fungal wheat pathogen

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Transposable elements (TEs) are key drivers of genomic variation contributing to recent adaptation in most species. Yet, the evolutionary origins and insertion dynamics within species remain poorly understood. We recapitulate the spread of the pathogenicity-associated Styx element across five species that last diverged ~11,000 years ago. We show that the element likely originated in the Zymoseptoria fungal pathogen genus and underwent multiple independent reactivation events. Using a global 900-genome panel of the wheat pathogen Z. tritici, we assess Styx copy number variation and identify renewed transposition activity in Oceania and South America. We show that the element can mobilize to create additional Styx copies in a four-generation pedigree. Importantly, we find that new copies of the element are not affected by genomic defenses suggesting minimal control against the element. Styx copies are preferentially located in recombination breakpoints and likely triggered multiple types of large chromosomal rearrangements. Taken together, we establish the origin, diversification, and reactivation of a highly active TE with likely major consequences for chromosomal integrity and the expression of disease.

Keywords: Transposable element, Genome evolution, Fungal pathogen, Structural rearrangement

*Speaker

Exploring transposable element-mediated adaptive trait evolution using a large fungal pathogen genome panel

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Transposable elements (TEs) are implicated in adaptation in several species, however identifying adaptive TE insertions remains challenging, often due to difficulties in confidently calling TE insertions from short-read data and a lack of detailed phenotypic information.

Zymoseptoria triciti is a globally distributed fungal pathogen of wheat, causing significant crop damage. TEs have caused genome size expansions in populations derived following the pathogen's spread from the centre of origin. Whilst some well-described examples of TE-mediated adaptation in *Z. tritici* exist, the significance of TEs in facilitating the species' global spread remains unknown. Rapid adaptation to new environments and increases in TE activity present an opportunity to systematically investigate the importance of TEs as a source of adaptive variation.

We manually curated 331 TE families using a pangenome assembly for *Z. tritici* from a panel of 19 reference-quality genomes. Leveraging 2,229 genomes sampled across the globe, we systematically explore the dynamics of TEs under positive selection and their involvement in adaptive trait variation.
We generated a high-confidence TE variant set by validating calls in benchmarking using validation from PacBio data. We find significant TE copy number expansions associated with specific populations, including large increases in LTR, DNA, and MITE activity in North American populations. In contrast to other organisms, pathogen population structure can be resolved using TE insertion polymorphisms. When assessing TE abundance, we find large numbers of population-specific TE loci at common frequencies, ranging from 91 in European isolates to 895 in Oceanian isolates. With comprehensive trait and climate data for our genome panel, we assess candidate polymorphic TEs for signatures of selection to determine their importance in the global spread of *Z. tritici*. Overall, this work sheds light on the host-TE dynamics leading to the emergence of adaptive traits, and the processes.

Keywords: adaptation, population genomics, annotation, pathogen evolution

*Speaker

Transcriptional regulation of dominance at the self-incompatibility locus in Arabidopsis

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Self-incompatibility (SI) mechanisms in hermaphroditic flowering plants serve as crucial barriers to self-fertilization. In the Brassicaceae, the locus governing SI displays remarkable diversity, featuring numerous distinct alleles retained over long evolutionary times and organized in a complex dominance hierarchy. Under this hierarchy, the gene controlling SI specificity in pollen exhibits monoallelic expression in heterozygote individuals. This is achieved through the action of sRNAs produced by precursors acting as "dominance modifiers" resembling miRNAs but compatible with multiple gene silencing pathways. Single sRNA precursor undergoes extensive processing, generating hundreds of sRNA molecules with varying sizes, abundance levels, and ARGONAUTE loading preferences. To study this gene silencing phenomenon, we established a reverse genetic approach in engineered *Arabidopsis thaliana* lines expressing components of the *A. halleri* SI system and observed that the transcriptional repression is independent of the canonical RNA-directed DNA Methylation pathway (RdDM). We developed a single-molecule transcript capture protocol, and remarkably we observed that the sRNAs seem to target the transcription start site, possibly indicating an interference with the transcriptional machinery. Overall, the question of the mechanisms by which this repression occurs remains open, especially regarding the role of DNA methylation on target sequences. Unraveling these silencing mechanisms will offer insights into the mechanisms by which dominance/recessivity interactions can evolve.

Keywords: Self, incompatibility, small RNAs, epigenetics

*Speaker

How genes endure intronic transposable elements.

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Transposable elements (TEs) compose most of the human genome and more than 45% of introns. A major part of intronic TEs are relics containing cryptic splicing, termination signals, and abundant DNA and RNA protein binding sites. As such, intronic TE sequences could interfere with transcription elongation of the genes in which they are integrated, through premature transcription termination, a process known as attenuation. As attenuation appears to be uncommon under control conditions, we hypothesized that safeguarding mechanisms might protect long human genes. To study such mechanisms, we developed an R package, called tepr, to analyze datasets from nascent transcript RNA sequencing (e.g., TT-seq). The software can identify genes with potential attenuation, measure the extent of attenuation, and compare different experimental conditions. Applying this strategy to human fibroblasts cells treated with heat-shock (HS), a condition known to induce attenuation on long genes (Cugusi et., 2022), we identified a specific set of 344 genes experiencing attenuation upon HS. To find potential cellular factors involved in promoting or preventing attenuation, we further screened publicly available eCLIP datasets and discovered RNA-binding proteins enriched in transcripts from HS-attenuated genes, suggesting that these factors could regulate attenuation. Altogether, this work provides a quantitative framework for studying attenuation and sheds light on the regulatory mechanisms protecting transcription elongation through long human introns. This work is supported by Agence Nationale de la Recherche (ANR-11-LABX-0028; ANR-15-IDEX-0001; ANR-19-CE12-0032), Fondation pour la Recherche Médicale (FDT202304016688) and CNRS GDR 3546.

Keywords: transcription, elongation, attenuation, intron, heat, shock, eCLIP, RNA binding, transposable elements, genes

*Speaker

Uncoupling programmed DNA cleavage and repair scrambles the Paramecium somatic genome

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In *Paramecium*, genome rearrangements involve the precise excision of numerous single-copy relics of mobile elements, called internal eliminated sequences (IESs), from somatic DNA. Rearrangements are initiated by DNA double-strand breaks (DSBs) requiring the endonuclease activity of a domesticated piggyBac transposase, PiggyMac (Pgm), and repaired by the classical non-homologous end joining (NHEJ) pathway. These two steps (DNA cleavage and repair) are tightly linked and RNA interference against *KU70* or *KU80c* (a specialised paralog of *KU80*) induces a developmental phenotype with complete inhibition of inhibition of DNA cleavage. Using a Ku70 DNA repair defective mutant, we have shown that coupling between DNA cleavage and repair machinery ensures faithful DNA repair of IES flanking sequences. In the absence of coupling, assembly of the new somatic genome leads to numerous errors, including ends repaired by de novo telomeric addition and numerous translocations between IES flanking sequences. We have investigated the role of other components of the NHEJ machinery encoded by the *Paramecium* genome. All are essential for the survival of the sexual progeny, but their depletion leads to very different molecular phenotypes. Depletion of Xlf or DNAPKcs inhibits DNA repair, whereas the absence of two different homologs of Paxx leads to IES retention, demonstrating that additional NHEJ factors besides Ku70/80 contribute to coupling. The properties of the DNA repair factor required for DNA cleavage and the nature of the errors observed under uncoupling conditions suggest a model for Pgm-dependent cleavage activation. In this model, the Pgm-domesticated transposase is activated by the formation of a synaptic complex based on DNA repair proteins.

Keywords: Domesticated transposes, NHEJ, Programmed genome rearrangements

*Speaker

Impact of Transposable Elements insertion on apple genome evolution

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The apple tribe is native to the Tian Shan mountains in Asia (1). The common ancestor of apple and pear underwent a whole genome duplication (WGD) that occurred during Himalayan chain formation. This WGD, dated 27 Mya (2), resulted in autopolyploid plants containing two identical subgenomes (3). Duplicated genes originating from WGD are named ohnologous genes (4). This WGD is considered as a genomic shock, which associated with major environmental changes due to ground elevation, may have led to a burst of transposable elements (TE) 21 Mya (5). We identified that QTLs in apple are not distributed evenly among ohnologous chromosomes. This imbalance has been associated with significant differences in the expression level of ohnologous genes in M. domestica (2).

Using 149 RNA-seq experiments derived from a wide array of apple cultivars, we performed differential expression analysis to compare ohnologous genes expression (2). We identified 828 ohnologous gene pairs for which one gene of the pair was systematically overexpressed relative to the other in all experiments. We named these genes "non switching".

In this project we focus on non switching pairs and compare them to a random sample of 828 ohnologous pairs with differential expression level varying throughout the RNA-seq experiments (called swiching genes).

Our objective is to identify the epi/genetic mechanisms that may explain the observed differential expression within the non switching group. The TE environment of the genes were analyzed using TEGRIP pipeline (6), which extract the TE insertion's position relative to the genes. An enrichment analysis was then performed to associate particular TE environment(s) (including TE composition and insertion's locus) with each gene classes. The results will inform us on the evolution of TE environment post WGD, and whether TE insertion nearby genes causes differential expression among ohnologous pairs.

Keywords: Apple, WGD, autopolyploid, chromosome dominance, evolution, Transposable Elements.

*Speaker

The role of transposable elements in the function and evolution of centromeres

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Transposable elements (TEs) represent the most abundant component of eucaryotic genomes and are key drivers of genomic and phenotypic evolution. Despite their importance, several aspects of their interactions with the host genomes are still not well understood. One of them revolves around their role in the function and evolution of centromeres, regions of ultra conserved function that can, however, rapidly evolve between species. Aided by recent advances in sequencing technologies, we recently showed that TEs are disruptive agents of the genetic and epigenetic environment of centromeres in Arabidopsis thaliana, and are engaged in cycles of invasion and purging through satellite homogenization - processes that drive centromere and genome evolution, and, ultimately, speciation. Taking it further, we now explore the role and dynamics of TEs in centromeres across the tree of life. This talk will cover recently published data, but also new findings from our ongoing work.

Keywords: transposable elements, centromeres, genome evolution

*Speaker

The potential role of transposable elements in the development of Schistosoma mansoni

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With the completion of a high quality genome for the human parasite Schistosoma mansoni, we have performed a thorough identification and annotation of transposable elements (TEs) within this non-model species. By manually curating the data, a library of 81 TE families was produced where we not only discovered novel families, but also redefined previously existing ones. As observed, we did not find any new class II elements apart from the three that were already described. The most recent expansion is observed in LINE and PLE elements, while LTR elements had an earlier expansion which has now decreased.
A structural analysis showed a concordant distribution among the different chromosomes, except for the sex specific part of the sexual chromosomes, with an accumulation in subtelomeric regions. Interestingly, we found TEs are as frequently present in intergenic regions as in gene bodies, where they are preferentially inserted in introns showing an increasing frequency towards the end of the gene.

Analysing expression of TEs along the life cycle, we observe different patterns that characterize each stage, including all Penelope elements being strongly expressed in the miracidia stage. We are currently investigating if the expression correlates with gene expression that correlates with the metabolic processes specific for each stage.
We also looked at differential expression in paired and unpaired adults. In males, we observe different families being expressed, but these changes remain moderate. When looking at paired versus unpaired females, we observe a drastic increase in expression of the majority of the TE families. This is significant as females undergo sexual maturation when paired and might indicate a possible role in the processes controlling sexual maturation in Schistosoma.

Keywords: Transposable element, expression, development, parasite

*Speaker

A comparative analysis of transposable elements in the subphylum Pucciniomycotina reveals a retrotransposon-driven expansion in the massive genomes of rust fungi

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Impact of transposable elements (TEs) on genome architecture and evolution stems from their mobility, which ultimately can lead to modifications in the genome including functional disruption and alteration in gene expression. In fungi, TEs genome coverage is extremely variable from one species to another. Rust fungi (i.e. taxonomical order Pucciniales) represent the largest group of obligate biotrophic plant pathogens in the Kingdom Fungi, with more than 8,000 estimated species. The Pucciniales are notorious for their extremely large genome size (from 80Mb to more than 1Gb compared to $<$ 50Mb on average in fungi) with higher gene and TE contents compared to closely related species within the subphylum Pucciniomycotina. For instance, the 1.2Gb diploid genome of the Asian soybean rust fungus, *Phakopsora pachyrhizi* is composed of $>$ 93% of TEs and contains more than 22,000 genes. Here, we investigate the landscape and evolutionary history of TE invasions in the genomes of 11 Pucciniales species compared to four species within the subphylum Pucciniomycotina. We used the REPET pipeline to detect and annotate TEs in order to determine the coverage and composition of TEs within Pucciniomycotina genomes. We found an enrichment of LTRs (Gypsy) and LINE retrotransposons in all genomes of rust fungi, indicating that these elements are the main contributors to genome size expansion in the Pucciniales. An in-depth analysis of retrotransposons insertion ages, based on applying LTR Kimura distance, revealed that most retrotransposons have proliferated less than two million years ago in the genomes of the Pucciniales. Our study indicates a shift in the genomic landscape of Pucciniales shaped by distinct transposable element activity during the recent history of Pucciniomycotina.

Keywords: Transposable Elements, Rust fungi, comparative genomics, bioinformatics

*Speaker

Transposable elements driven adaptation to temperate environments in the Asian tiger mosquito Aedes albopictus

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Understanding how species and populations adapt to new environmental conditions is one of the goals of evolutionary biology. The NGS revolution made possible mapping the footprint of natural selection in genomes at a very fine resolution, yet the contribution of transposable elements (TEs) to adaptation has often been ignored but in a few model systems. Studying the mechanisms by which invasive species thrive in new environments can illuminate our understanding of adaptation in general. The Asian tiger mosquito Aedes albopictus is one such species. This mosquito is a vector of several arboviruses such as Dengue and Chikungunya viruses. From its cradle in Asia it has spread to Europe, the Americas and Africa thanks to the trade of used tyres in the last 40 years. One of the most conspicuous characteristics of this mosquito is the ability of temperate populations to produce cold-hardy diapausing eggs in response to shortening days to ensure winter survivorship. While this adaptive phenotype has probably been acquired during the colonization of Northern parts of Asia from tropical South-east Asia a few 10,000s years ago, there is evidence of rapid adaptive evolution of the photoperiod induced diapause in recently invaded areas. Another remarkable aspect of this mosquito is that its genome is riddled with TEs ($> 60\%$ of the genome) which may represent a huge reservoir to produce genetic variation. Here we present a project aimed at uncovering the contribution of TEs to adaptation in Aedes albopictus, with a special emphasis on its recent adaptation to temperate environments. This will be achieved first by detecting TE insertions that display the footprint of adaptation in the Aedes albopictus 1200 genomes project and then by characterizing the molecular and macroscopic phenotypes conferred by these insertions.

Keywords: Population genetics, Aedes albopictus, adaptation

*Speaker

LTR- Retrotransposon dynamics during the polyploidization and domestication of cotton

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Gossypium hirsutum and *Gossypium barbadense* are two allopolyploid species of cotton that have undergone two parallel domestication processes and represent the world's largest source of natural fiber. Recent research has provided good quality reference genomes of 8 and 11 different accessions of *G.hirsutum* and *G. barbadense* respectively, including both wild and domesticated populations. The current availability of such multiple high-quality reference genomes enables the development of reference pangenomes for genomic and evolutionary studies, which significantly improves the capture of the genetic diversity within a species in comparison to single reference genomes. With the aim of elucidating the roles of TEs in both the allopolyploidization and domestication events of these two species, we have analysed the LTR-RT content within the available *G. hirsutum* and *G. barbadense* genomes and its diploid parental species. Moreover, we have generated species-level pangenomes that allow us to study the more recent TE dynamics by looking into the structural variants triggered by TEs, also called TIPs (Transposon Insertion Polymorphisms). Our preliminary results reveal that different LTR-RT lineages show different dynamics after polyploidisation, with some lineages increasing their copy number accompanying the polyploidization event and others showing a relatively low activity and maintaining their copy numbers. Moreover, our results also show that the two tetraploid species seem to have undergone different LTR-RT recombination dynamics, evidenced by the presence of solo-LTR TIPs. These results illustrate how diverse the LTR-RT dynamics can be even within a single genome and how LTR-RT amplification and elimination by intra-element recombination shape a complex genome such as that of cotton.

Keywords: LTR, retrotransposon, cotton, TE dynamics, polyploidy

*Speaker

Transposon insertions associate with transcriptional variability linked to rice domestication and breeding

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TEs account for a large fraction of the genetic variation found in crops. Numerous studies have reported examples of TE insertions regulating agronomic traits, usually by altering transcriptional levels of nearby genes. TEs have been recently active in rice (*Oryza sativa*), and young insertions are known to contribute to genetic and phenotypic variability. Nevertheless, the role that TE standing variation present in the rice ancestors (*O. rufipogon* and *O. nivara*) has played during rice domestication and breeding is unknown. In this study, we detected TE polymorphisms (TIPs) in a population of rice and their wild relatives (n = 290 accessions). We looked for TIPs linked to gene expression variation by performing TIP-eQTL mapping using transcriptomic data of the rice (indica and japonica) accessions. We found 829 TIPs associated with expression changes in cis (5Kb cut-off distance from gene). These insertions were enriched in promoter regions and often explained more transcriptional variance than SNP-eQTLs, suggesting that they could frequently be the causal mutation. Up to 66% of these TIPs were found in the population of wild relatives (n = 82 accessions), suggesting that they were present in the rice recent ancestor. Further analyses of the TIP-eQTLs revealed that they often affect genes related with domestication traits and had undergone differential patterns of selection on indica and japonica subspecies. Our results suggest that TE insertions inducing expression changes on signal transduction genes have been maintained in wild rice populations and have been selected during the domestication and adaptation of rice populations

Keywords: Transposon Insertion Polymorphisms, transcription, traits, positive selection

*Speaker

Can repetitive elements lead to speciation through chromosomal rearrangements?

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Large-scale chromosomal rearrangements may act as reproductive barriers, contributing to speciation. It has been hypothesised that chromosomal rearrangements are caused by repetitive elements such as transposons. Lepidoptera, i.e. butterflies and moths, which are among the most diverse groups across the Tree of Life, have holocentric chromosomes without defined centromeres. Chromosomal fusions and fissions are thought to be less deleterious in holocentric organisms and therefore more likely to be retained. Accordingly, some butterfly clades show a tremendous diversity of chromosome numbers. These clades often show bursts of species diversity, suggesting a role of rearrangements in speciation. To advance our understanding on the importance of repetitive elements and chromosomal rearrangements in speciation, we have generated and compared chromosome-scale genome assemblies for *Erebia* butterflies with differing chromosome numbers. We found support for a role of some repetitive elements in chromosomal rearrangements. Therefore, differences in repeat expansions might explain why some clades show a greater variation in chromosome numbers and a greater species diversity than others. Overall, we are disentangling the association between repetitive elements, chromosomal rearrangements, and species diversification in holocentric organisms.

Keywords: speciation, chromosomal rearrangements, structural variants, transposable elements, Lepidoptera

*Speaker

Epigenetic regulation of repetitive DNA in insect Tribolium castaneum

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Repetitive DNA constitutes a substantial portion of eukaryotic genomes, but is often absent from genome assemblies because its repetitive nature causes difficulties during assembly process. With the development of long-read sequencing, these regions are becoming increasingly accessible for in-depth research. Despite advances in genomics, the epigenetic regulation of repetitive DNA remains largely unexplored. In our study, we investigated the intricate landscape of transposable elements (TEs) and satellite DNA (satDNA) and their influence on neighboring regions in the insect model organism *Tribolium castaneum*. First, we utilized nanopore sequencing unique ability to detect methylated DNA bases to study epigenetic modifications of repetitive DNA. To further decipher epigenetic regulation, we explored mechanisms at the protein level by performing ChIP-seq experiments and targeting various active and repressive histone marks corroborated by immunostaining with different histone antibodies. Additionally, to investigate the presence of non-coding RNAs with regulatory function we performed small RNA sequencing experiments. Surprisingly, our results show the absence of 5-methylcytosine and extremely low levels of N6-methyladenine modifications, indicating that these epigenetic markers probably do not affect biological processes in the genome of *T. castaneum*. In contrast, histone modifications analyses indicate a possible regulatory role of these modifications in epigenetic control of TEs and satDNA. Transcriptional analysis showed developmentally regulated patterns and differentially expressed repetitive DNA. Furthermore, small RNA examination indicated a preferred sequence length originating from the most conserved part of the repetitive elements highlighting the possibility of underlying regulatory mechanisms. Our comprehensive study has provided novel insights into epigenetic mechanisms of repetitive DNA regulation in *T. castaneum*, laying the foundation for future investigations into their impact on gene regulation and overall genome dynamics.

Keywords: repetitive DNA, epigenetics, methylation, transcription, Tribolium

*Speaker

De novo identification of Transposable Elements from RNA-seq data

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Short-read RNA sequencing has generated extensive collections of reads. The goal of our work is the de novo identification of repetition families from RNA-seq reads. This would help to discover novel repetition families, including transposable elements, in particular for non-model species. This could also help to improve de novo transcriptome assembly.

We are specifically working with De Bruijn graphs, an efficient data structure where every transcript corresponds to a path within this graph. Our research involves characterizing complex regions that contain families of repetitions and replacing them with consensus nodes. The objective of this novel method is to operate de novo, without relying on genomic references nor repeat consensus sequences.

Preliminary results in dog and drosophila datasets have enabled us to identify regions of the De Bruijn graph that are associated with various types of repetitions. Some of these repetitions are TEs. Out of those, we expect that some correspond to full-length active families, while others are TE-derived elements associated with TE insertions within genes.

Keywords: De Bruijn graph, repetition family, RNA, seq

*Speaker

Integrons are goldmines of anti-phage defense systems

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Integrons are genetic platforms that allow bacteria to adapt to different environmental stressors through the acquisition of new genes embedded into cassettes. These systems work by capturing, storing and rearranging cassettes, which ensures the exploration of diverse phenotypic combinations as only the first cassettes of the integron are expressed (1). While integrons are best known for their role in antibiotic resistance, up to several hundred uncharacterised cassettes can be found in large integrons. Given the plasticity of these adaptation on demand platforms, in this work we set out to explore the role of the cassettes present in the *Vibrio cholerae* chromosomal integron as defence systems against phages. Through the overexpression of 88 of these cassettes, we found that 16 displayed anti-phage activity in either *V. cholerae* or *E. coli*. We also showed that these usually silent cassettes can be mobilized and expressed by the integron and thus confer resistance to phages in their natural genetic context. Most of the systems encoded in these cassettes have little or no similarity to previously known ones. Moreover, they exhibit typical features of abortive infection behavior, in which the defense system targets its host cell after detecting the presence of the phage, preventing viral amplification and protecting the rest of the population (2). With these results, we confirm the propensity of integrons to be hotspots for anti-phage systems at a low cost for the cells.
(1) Escudero, Loot, Nivina and Mazel, *Microbiol Spectr*, Mar. 2015
(2) Yen *et al.*, *Nat. Commun.*, Feb. 2017

Keywords: integrons, bacteriophages, phage defense, recombination

*Speaker

Mode, tempo, and functional consequences of transposable element mobilization in selfing and outcrossing Arabidopsis lyrata populations

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Transposable elements (TEs) are now widely recognized as major contributors to genome evolution, yet the processes governing their accumulation remain elusive. Mating systems are expected to play a central role, but the effect of shifting from outcrossing to selfing, which occurs commonly in plants, is not known. To study how mating systems impact the dynamics of TE accumulation, we used the outcrossing species Arabidopsis lyrata which also has some North American populations that have recently experienced a shift to selfing. Specifically, we have sequenced from both selfing and outcrossing populations 20 genomes using long reads Oxford Nanopore Technology as well as 19 additional ones using short read Illumina sequencing. Using the short reads sequencing data, we have characterized the impact of selfing on genetic diversity as well as on the efficacy of purifying selection at the Single Nucleotide Polymorphism (SNP) level. After assembling the long-read sequenced genomes de novo, we have then detected transposable element insertion polymorphisms (TIPs) across these populations. We will discuss results from these analyses and how they inform us on the contribution of TEs to genetic diversity in relation to mating systems.

Keywords: mating systems, transposable element insertion polymorphisms, genetic diversity

*Speaker

Antagonistic coevolution of telomeric TEs and their host across the Drosophila genus

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One of the most unusual and striking examples of an active TE benefitting its host genome involves the telomeric transposons of *Drosophila*. All *Drosophila* species lack telomerase and instead have telomeres composed of head-to-tail arrays of specialized retrotransposons. These TEs are from the Jockey clade of LINE retrotransposons and were first characterized in *D. melanogaster*, where 3 telomeric TE families were identified: *Het-A*, *TAHRE*, and *TART*. We previously showed that *TART* captured a portion of the host piRNA pathway gene *nxf2*. Antisense piRNAs are produced from the *nxf2*-like region of *TART*, which target their cognate host gene for silencing. This strategy represents a novel form of counter-silencing by TEs. Here, we have surveyed long-read genome assemblies of over 100 species of *Drosophila*, identifying a total of 372 telomeric TE families, including two completely novel clades. Surprisingly, we find that capture of piRNA pathway gene fragments has occurred independently at least 8 times across the genus, suggesting that this counter-silencing strategy is relatively common among these TEs. More generally, we show that these telomere specialized elements evolve rapidly and dynamically. For example, we find that the telomeric TE gene tree is highly discordant with the host species tree, which is best explained by a large number of horizontal transfer events, as well as TE family extinction. In fact, we find that telomeric TEs have been lost completely at least 10 times across the *Drosophila* phylogeny. We additionally discover a novel ORF, which has replaced the *POL* gene in a subset of these elements, as well as an instance of convergent evolution of telomere specialization. These results provide unprecedented detail into the evolution of these unusual TEs and highlight several novel mechanisms by which they evolve in conflict with their host genome despite the essential telomere function they provide.

Keywords: Drosophila, telomeres, evolution, piRNAs

*Speaker

The role of ORF1p in LINE-1 Retrotransposition

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Retrotransposons are genetic elements able to self-replicate via a "copy and paste" mechanism that requires reverse transcription of an RNA intermediate, and insertion of the cDNA copy within the genome. The only autonomously active retrotransposable element in humans is the long-interspersed element 1 (LINE-1). Notably, LINE-1 fragments comprise 17% of the human genome and have been implicated in many human diseases due to generation of pseudogenes and chromosomal rearrangements. Full-length, active LINE-1 transcripts are 6kb bicistronic mRNAs that encode the proteins ORF1p and ORF2p which are required for retrotransposition of the LINE-1 transcript. ORF1p and ORF2p interact with the LINE-1 transcript to form a ribonucleoprotein (RNP). ORF2p has been well characterized as a multifunctional protein with endonuclease and reverse transcriptase activity, whereas ORF1p is an RNA-binding protein with mRNA chaperone activity, but its role in coordinating the LINE-1 lifecycle remains elusive. Additionally, overexpression of ORF1p has been found to be a hallmark of many cancers and neurodegenerative diseases. However, a mechanistic role of ORF1p in these diseases is unclear. Results from preliminary work suggest that ORF1p forms phase separated condensates that can enter the nucleus to carry out retrotransposition. These condensates exhibit correlated motion and co-localization with mitotic chromatin in cells. This finding, along with live-cell observations of lagging chromosomes and anaphase bridges associated with nuclear ORF1p condensates, suggests a new function for ORF1p- DNA-binding. Preliminary data from DNA-curtains not only confirms DNA-binding activity of ORF1p but also cross-linking of multiple DNA strands, suggesting a potential role for the protein in LINE-1-mediated genomic instability. Using a unique approach combining high-resolution live cell imaging, single-molecule DNA curtain technology, and single-cell DNA sequencing, I aim to determine the contribution of ORF1p to the LINE-1 lifecycle, including its interactions with DNA, mitotic chromatin, and its involvement in LINE-1-mediated disease pathogenesis.

Keywords: LINE1, ORF1p, DNA curtains, DNA binding, genome instability

*Speaker

Characterizing the transposable element landscape of the vertebrate water-to-land transition

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The vertebrate water-to-land transition represents a pivotal event in the history of life on Earth. In order to adapt their body plan to terrestrial environments, early tetrapods must have undergone changes in their gene regulatory landscape. It is now well accepted that transposable elements (TEs) contribute to evolutionary processes by providing and mobilizing cis-regulatory elements (CREs). Thus, our work aims to explore the role of TEs in the evolution of the adaptations that enabled the water-to- land transition in vertebrates.

We began by generating repeat annotations for lobe-finned fishes – closest extant relatives of tetrapods – and amphibians, representing an early branching tetrapod group. Intriguingly, lungfishes and salamanders possess giant genomes between 20 and 132 Gbp in length, and it remains to be determined whether their genomic gigantism reflects a higher rate of transposition or a failure to purge ancient repeats. If this second possibility were the case, it would allow us to explore the origins of ancient CREs and determine to what evolutionary innovations might have arisen thanks to the action of TEs.

Unfortunately, genomic giantism represents a major technical challenge in terms of annotating their TE landscape with existing tools. Thus, we developed a suite of TE annotation pipelines able to handle extreme genome sizes. Following de novo repeat library generation, our toolkit performs consensus extension and incorporates searching through highly-sensitive HMM models. It also identifies orthologous TEs insertions across species and thanks to its efficiency and scalability, we have deployed it across a collection of lobe-finned fish and tetrapod genomes in order to explore the TE dynamics of the water-to-land transition.

Through this comprehensive exploration of TE involvement in the water-to-land transition, we anticipate shedding light on the fascinating TE biology and deepening our understanding of their significance in driving the emergence of evolutionary innovations.

Keywords: Amphibians, TE, Annotation, Salamanders, Axolotl

*Speaker

TEFLoN2, an automized and accurate computational tool for detecting and analyzing insertions of transposable elements in population data

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Transposable elements (TEs) are mobile, repetitive and mutagenic elements of DNA known to be important components of eukaryotic genomes and major actors in genome evolution. In order to estimate their impact on genome evolution, we need to detect them from individual to populations. Detection of TE insertions using paired-end reads revealed a high false-positive rate, up to 40%. Even if long–read sequencing technologies overcome this detection issue, this type of sequencing is not suitable for population genomics studies that require a large amount of resequencing data from multiple samples and for a reasonable cost.
One of the most promising tools highlighted in previous benchmark studies is TEFLoN that uses short-read pooled-data. While TEFLoN is easy to install and use, many technical limitations have been identified, such as the fact that each script must be launched independently without parallelization that makes it time and memory consuming. Thus, we developed an automatic and optimized version of this tool called TEFLoN2 by upgrading the code and developing a SnakeMake pipeline. We also developed a new module accurately estimating the TE frequency in pooled or large single sequencing dataset. We were then able to appreciate its accuracy in simulated and public resequencing data (https://github.com/asfistonlavie/TEFLoN2).

Keywords: Transposable elements, structural variants, annotation, snakemake pipeline, population genomics

*Speaker

The causes and consequences of low TE content in African mole-rats

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Aside from their exceptionally long lifespans and resistance to aging-related diseases, female African mole-rats (Rodentia: Bathyergidae) are fertile for most of their life. As transposable elements (TEs) have been previously implicated in aging and inflammatory responses, we would like to explore possible connections between the TE content of African mole-rat oocytes and their unusual reproductive longevity. In this study, we first compared the TE content of three African mole-rat species (naked-mole rat, Mechow's mole-rat and Damara mole-rat) to three other rodent species (mouse, Upper Galilee Mountains blind mole rat and guinea pig) by creating two sets of TE libraries/annotations: one with all TEs using RepeatModeler and RepeatMasker, and another specifically for LTR-retrotransposons (LTR-RTs) using LTR_retriever. We used two genome assemblies for naked mole-rat: its reference genome generated with short-read data, and a newer chromosome-level assembly created with long reads. As in previous studies, we found the number of total and young TE and LTR-RT sequences in African mole-rats to be lower than in mouse. However, we observed a higher number of LTR-RTs and a more recent LTR-RT acquisition peak from the new naked mole-rat assembly compared to the reference genome. We then compared rates of LTR-RT removal through ectopic recombination between mole-rats and other rodents. While Mechow's mole-rat had the highest ratio of solo LTR versus intact LTR-RTs, Damara mole-rat and naked mole-rat had the lowest and second lowest, respectively. Our results suggest that although African mole-rats have relatively fewer total and young TE insertions, recombination-based LTR-retrotransposon removal is not conserved in African mole-rats and cannot fully explain their low LTR-RT content. Furthermore, reported TE content and age can vary depending on the quality of the genome assembly and the TE detection method used.

Keywords: African mole, rats, rodents, oocyte, reproductive longevity, LTR retrotransposons

*Speaker

RepetDB

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Transposable elements (TEs) are major players in the structure and evolution of eukaryote genomes. Thanks to their ability to move around and replicate within genomes, they are probably the most important contributors to genome plasticity. The insertion of TEs close to genes can affect gene structure, expression and function, contributing to the genetic diversity underlying species adaptation. Many studies have shown that TEs are generally silenced through epigenetic defense mechanisms, and that these elements play an important role in epigenetic genome regulation. Their detection and annotation are considered essential and must be undertaken in the frame of any genome sequencing project. Here, we will present the new version of RepetDB (1) (Amselem et al., Mobile DNA, 2019), (https://urgi.versailles.inrae.fr/repetdb) our TE database developed to store and retrieve detected, classified and annotated TEs in a standardized manner. This RepetDB v2 new version was updated with more species of plants and fungi and provides TE consensi with evidences able to justify their classification. RepetDB v2 is a customized implementation of InterMine (2,3), an open-source data warehouse framework used here to store, search, browse, analyze and compare all the data recorded for each TE reference sequence. InterMine provides powerful capabilities to query and visualize all biological information on TE. It allows to make simple search on the database using the QuickSearch ('google like search') or make more complex queries using the Querybuilder to display various desired information. RepetDB v2 is designed to be a TE knowledge base populated with full de novo TE annotations of complete (or near-complete) genome sequences. Indeed, the description and classification of TEs facilitates the exploration of specific TE families, superfamilies or orders across a large range of species. It also makes possible cross-species searches and comparisons of TE family content between genomes.

Keywords: Database, consensus, Tool, transposable elements

*Speaker

Specialized group II intron retrotransposons

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1

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Group II introns are large catalytic RNAs (ribozymes) and retrotransposable elements of bacterial origin. They invade genomes by 'retrohoming', a highly site-specific mobility pathway that is operated both by the intron ribozyme and the intron-encoded reverse transcriptase. Most often, mobile group II introns insert themselves into intergenic regions of the genome, which is consistent with the 'selfish' behavior of these mobile elements. In contrast with this trend however, some classes of group II introns target particular structures or genetic contexts. This is the case of some particular group II introns that are found associated with 'start' or 'stop' codons of specific genes. These 'specialized' introns, abundant in Gram-negative bacteria, also share peculiar structural features. To study the biology of these retrotransposable elements we are using as a model system an *E. coli* group II intron retrotransposon whose natural integration site corresponds to the 'stop' codon of the heat-shock gene *groEL*. Using genetic and molecular approaches, we have shown that this 'groEL' intron is a highly mobile and site-specific retrotransposon. Interestingly, we have also demonstrated that the 'groEL' element can invade an ectopic copy of its target site, engineered in a transcriptionally silent locus of the host chromosome, as efficiently as the authentic target site at the end of the groEL ORF. To further dissect the mobility mechanism of this retrotransposon, we are currently testing the importance of a specific base-pairing interaction between the retrotransposon RNA and its DNA target on the ability of the 'groEL' element to integrate into its target site. In parallel, we have constructed several *E.coli* laboratory strains carrying a genomic copy of either the complete retrotransposon or different structural variants of it. These strains are being characterized phenotypically in order to uncover a potential role for the 'groEL' element in the physiology of the host cell.

Keywords: group II intron, bacterial retrotransposon, mobility mechanism, bacteria

*Speaker

Impacts of arboviral infections on transposable element transcript levels in Aedes aegypti.

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Transposable elements (TEs) are mobile and repeated sequences found in all genomes. TEs are controlled by RNA interference pathways in most organisms, and this control involves specifically the piRNA pathway, but also the siRNA pathway, which is also known to be the first line of antiviral defense in invertebrates. Using Drosophila, we have recently shown that viral infections resulted in the modulation of TE transcript amounts through modulations of the small RNA repertoires. The Aedes aegypti mosquito is of particular interest because almost half of its genome is made of TEs and it is described as a major vector of viruses (such as dengue (DENV), zika (ZIKV) or chikungunya (CHIKV) arboviruses). Moreover, Aedes mosquitoes are particular among insects in that the piRNA pathway is also involved in somatic antiviral response in addition to TE control and piRNA pathway genes expanded in the mosquito genome. For all these reasons, we decided to study the impacts of viral infections on TE transcript amounts in Ae. aegypti samples. We retrieved public datasets corresponding to RNA-seq data obtained from viral infections by DENV, ZIKV and CHIKV in various tissues. We find that TE transcripts are moderately modulated following viral infection and that the direction of the modulation largely varies across tissues and viruses. These results reinforce the need for a deep investigation of the tightly intertwined interactions between TEs and viruses.

Keywords: mosquito, DENV, CHIKV, ZIKV, retrotransposon, TE control

*Speaker

Genomic Responses to Interspecific Hybridization in Drosophila: Insights into Gene Expression, Transposable Elements, and Hybrid Sterility

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Interspecific hybridization is considered a genomic stress resulting in new gene expression patterns and transposable elements (TE) deregulation. Understanding expression changes in hybrids *vs* parental species is essential to unravel their potential role in speciation processes. The hybridization between *Drosophila buzzatii* and *D. koepferae* species produces sterile males and fertile females that can be backcrossed (BC) with parental *D. buzzatii* males, gradually restoring male fertility in the offspring from the third BC generation onwards. The main goal of this project is to identify the key genes involved in hybrid male sterility and the TE activity over different generations of backcrossing until male fertility recovering. We studied the testicular transcriptome of parental species, F1 and four generations of backcross hybrids. We observed that 26% of genes were deregulated in F1 hybrids *vs* parental species and all those located on the Y chromosome showed underexpression *vs D. buzzatii* in F1 and BC1 hybrids.
4.8 and 0.7 % of TE copies were differentially expressed in F1 hybrids *vs D. buzzatii* and *D. koepferae* respectively, with a general trend towards underexpression. The total number of copies from parental species differentially expressed in backcrosses decreased, but most of the *D. buzzatii* ones were overexpressed, except in BC1. Some piRNA pathway genes, involved in TE silencing, showed a deregulation in F1 *vs* both parental species and is maintained until BC1 in some of them, with Fs(1)Yb being the only gene exhibiting deregulation in all crosses. Finally, 21% of the genes differentially expressed in parental species have specific TE insertions within or their vicinity.
This work provides the first comprehensive transcriptomic study of Drosophila male interspecific hybrids, including the Y chromosome. It gives us important insights into how deregulation of gene and TE expression, mediated by genomic stress, can affect hybrid sterility.

Keywords: Drosophila, interspecific hybrids, deregulation, transposable elements, male sterility

*Speaker

Investigating the role of transposable elements in human brain evolution

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The human brain is the largest and most complex of all primates, having increased about 3-fold in size compared to that of our closest living relative, the chimpanzee. Little is known about non-human primate brain development as non-human primate tissue is not readily available at early developmental time points. Yet, it is essential to analyse non-human primate models to identify and understand molecular features unique to the human brain and its function. As only few changes occurred in protein-coding genes, significant differences must be present in other parts of the genome.
Transposable elements (TEs) comprise at least 50% of most primate genomes. They can contribute to genome evolution as they carry regulatory sequences and have been found to serve, for example, as enhancers and alternative promoters. Moreover, TEs are insertional mutagens leading to genetic disease. Therefore, they are silenced by repressive histone marks and DNA methylation. Interestingly, methylation can spread and impact adjacent regions making TEs noteworthy candidates for methylome evolution. However, due to their challenging analysis and lack of non-human primate material, the role of TEs in primate brain development and human brain evolution has not been investigated thoroughly.
Here, we exploit human, chimpanzee and rhesus macaque induced pluripotent stem cells and cerebral organoids as a model for primate brain development. We employ bulk and single-nuclei RNA-seq as well as Oxford Nanopore Technologies long-read whole genome DNA sequencing to investigate differential TE and gene methylation along with expression during neural differentiation in human and non-human primates. Using this multi-omics approach, we aim to identify species-specific TE loci important for brain development and evolution.

Keywords: human brain evolution, primate brain development, methylation

*Speaker

The impact of transposition on the evolvability potential of a fungal plant pathogen

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Transposition can impact genome structure and gene expression, resulting in phenotypic changes for selection to act on. Unrestricted transposable element (TE) activity can compromise genomic integrity and decrease population fitness. In eukaryotes, epigenetic modifications, including DNA methylation, help regulate genome stability through repression of TEs. However, the strong repression of TEs could impede the rise of beneficial genetic variation and constrain the evolvability of a biological system. In the case of the wheat fungal pathogen *Zymoseptoria tritici*, the inactivation of the DNA methyltransferase, Dim2, has resulted in near-complete loss of cytosine DNA methylation associated with an increased transcription of TEs. Interestingly, related species and *Z. tritici* strains from the pathogen centre of origin still possess an intact copy of the Dim2 gene. We aim to understand whether the loss of TE repression is adaptive and how it affects the evolutionary potential of *Z. tritici*. We selected eight *Z. tritici* strains naturally containing an active or inactive dim2 gene copy to generate Dim2 deletion and complementation transformants. By phenotyping the wild-type and mutant lines we have determined that absence of Dim2 and therefore TE silencing, has no immediate effects on *Z. tritici* fitness. We will now explore if the loss of dim2-dependent TE silencing confers evolutionary advantage in *Z. tritici* strains by evolving the wild-type and dim2 mutant strains for 52 weeks under optimal or stressful conditions. The genomes of the parental and evolved lines will be sequenced to search for de novo TE variants and their relative frequency will be correlated with the fitness of the evolved lines. The combination of experimental evolution, genomics, and epigenomics will highlight the significance of TE mobility in the evolvability of a wheat pathogen and facilitate further exploration of the role of TEs in the adaptability of organisms to stressful environmental conditions.

Keywords: fungi, plant pathogen, experimental evolution, evolvability, adaptation, DNA methylation

*Speaker

GraffiTE: a Unified Framework to Analyze Transposable Element Insertion Polymorphisms using Genome-graphs

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Transposable Elements (TEs) are abundant and mobile repetitive DNA sequences evolving within and across their hosts' genomes. Active TEs cause insertion polymorphism and contribute to genomic diversity. Here, we present GraffiTE, a flexible and comprehensive pipeline for detecting and genotyping polymorphic mobile elements (pMEs). By integrating state-of-the-art SV detection algorithms and graph-genome frameworks, GraffiTE enables the accurate identification of pMEs from genomic assemblies and long-read as well as the precise genotyping of these variants using short-or long-read data. Performance evaluations using simulated and benchmark datasets demonstrate high precision and recall rates. Notably, we demonstrate the versatility of GraffiTE by analyzing the human reference pangenome, 30 *Drosophila melanogaster* genomes, and multiple cultivars of the emerging crop model *Cannabis sativa*, where pMEs are undocumented. These analyses reveal the landscapes of pMEs and their frequency variations across individuals, strains, and cultivars. GraffiTE provides a user-friendly interface, allowing non-expert users to perform comprehensive pME analyses, including in models with limited TE prior knowledge. The pipeline's extensible design and compatibility with various sequencing technologies make it a valuable integrative framework for studying TE dynamics and their impact on genome evolution. GraffiTE is freely available at https://github.com/cgroza/GraffiTE.

Keywords: Transposable elements, insertion polymorphism, graph, genomes, structural variation, genotyping, genome evolution

*Speaker

Modeling the role of transposable elements in the adaptation and evolution of host genomes

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The abundance and variety of TEs within mammalian genome and their well-documented co-opted functions implies that these elements have, in part, driven the evolution of host genomes. However, a complete picture of the mechanisms governing this co-evolution as well as an understanding of the extent to which our genomes have been shaped by TEs is lacking. A major hurdle in comprehensively charting the co-evolution of host genomes with these friendly foe lies in the expanse of past and future evolutionary time that we cannot see. While ancient transposons which may have shaped the genomes of our ancestors are now decayed, often beyond recognition, the youngest, full length TEs are still active which means that, posing a threat to genome integrity, they must be silenced by the host. It is likely that these then remain far from being co-opted. To address this, we have established a model of transposon-host genome evolution in human stem cells; by introducing a novel transposable element to human cells – a young and active mouse TE IAPEz element – we are modelling TE invasion, fixation, regulation and co-option. Utilising comparative epigenomics, single cell locus specific TE-sequencing and single cell transcriptomics we are exploring the potential for host cells to use TEs to adapt under selective pressure and comprehensively cataloguing the genetic and epigenetic mechanisms via which this occurs. Our data suggests that over an adaptive time-course cells may utilise novel or "unannotated" enhancer regions which are enriched for LTR-transposons, implicating these TEs as being poised for their consequent co-option as cis-regulatory elements under the need to adapt to external stress.

Keywords: Evolution, Epigenetics, Neural Stem Cells, Single Cell transcriptomics

*Speaker

Universal signatures of transposable element compartmentalization across eukaryotic genes

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The evolutionary mechanisms shaping the origins of genome architecture remain poorly understood but can now be assessed with unprecedented power due to the abundance of genome assemblies spanning phylogenetic diversity. Transposable elements (TEs) are a rich source of large-effect mutations since they directly and indirectly drive genomic structural variation and changes in gene expression. Here, we demonstrate universal patterns of TE compartmentalization across eukaryotic genomes spanning ∼1.7 billion years of evolution, in which TEs colocalize with gene families under strong predicted selective pressure for dynamic evolution and involved in specific functions. For non-pathogenic species these genes represent families involved in defense, sensory perception and environmental interaction, whereas for pathogenic species, TE-compartmentalized genes are highly enriched for pathogenic functions. Many TE-compartmentalized gene families display signatures of positive selection at the molecular level. Furthermore, TE-compartmentalized genes exhibit an excess of high-frequency alleles for polymorphic TE insertions in fruit fly populations. We postulate that these patterns reflect selection for adaptive TE insertions as well as TE-associated structural variants. This process may drive the emergence of a shared TE-compartmentalized genome architecture across diverse eukaryotic lineages.

Keywords: genome evolution, origins of genome architecture, genome compartmentalization, comparative genomics

*Speaker

Hicberg – Hi-C Biological Estimation of Reapeated elements in Genomes

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During their evolution, the genomes of micro-organisms can acquire quantities of different repeated
elements such as retrotransposons, duplicated genes or tandem repeats. This type of sequence within
genomes cannot be processed directly by NGS technologies because they generate short reads that cannot be
located unambiguously on reference genomes. This information is filtered out by most current pipelines,
leading to incomplete genomic tracks resulting in a significant loss of information on biological functions,
processes and genomic structures involving repeated elements.
We developed hicberg, an algorithm that uses statistical inference and pseudo-random generators to predict
the positions of repeated sequence reads from different omics paired-end data (including Hi-C, Mnase-seq or
ChIP-seq). After developing the method and calibrating it on a test bench, we explored during this PhD
project how it improves genomic data interpretability of various species, starting with microbial ones such as
Saccharomyces cerevisiae. Reconstruction of Hi-C and ChIP-seq genomic tracks with hicberg revealed how
some retrotransposons in this model organism contribute in the positioning of cohesin, a molecular motor
involved in the formation of chromatin loops. A new role for retrotransposons sequences as contact points
for the elusive yeast 2 micron episomal molecule was also identified. Overall, these results underline the
power of the approach to discover new novel molecular relationships, and the interest in applying this tool
more widely to larger genomes with greater quantities of repeats. The proposed method can therefore
provide an alternative visualization of genomic signals in a wide variety of biological conditions and allow a
more comprehensive view of genome organization and plasticity. Importantly, existing datasets can be
revisited using the approach to unveil overlook features.

*Speaker

Keywords: Hi, C, Yeast, Ty, Computational biology

Tourist_15 – a Carrot MITE Family Rewires the Circadian Clock Regulatory Network by Redistribution of LHY Binding Sites

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One of the prominent features of transposable elements (TEs) is their ability to affect expression patterns of host genes. Upon insertion, they can provide novel *cis*-regulatory elements (CREs). Plant circadian clock regulatory network coordinates a range of important growth and cellular processes. *LATE ELONGATED HYPOCOTYL (LHY)* is one of the key regulators of the circadian clock in plants, also involved in abiotic stress responses. We searched for carrot MITE families carrying CREs. *Tourist_15* family members were highly enriched in LHY binding sites. Using DNA affinity purification sequencing (DAP-seq), we identified 11,779 LHY peaks genome-wide, of which 20% were localized in promoters. Of 2,346 genes with DAP-seq peaks within promoter regions, 74% were expressed and of those, the expression of 694 genes was affected by at least one abiotic stress.

We overlapped the experimentally identified LHY binding sites with the location of MITEs. We revealed that 1,428 (12%) of DAP peaks overlapped with MITE copies and 591 (41%) of those were within the *Tourist_15* family, comprising 56% of all copies. Positions of 163 and 73 *Tourist_15* copies bound by LHY were directly upstream or downstream the nearest gene, respectively, 35 and four were located in introns and cds, respectively, while the remaining 316 were intergenic. Expression levels of 203 genes (55% of all genes potentially controlled by CREs provided by *Tourist_15*) was affected by abiotic stresses. Of those, 123 *Tourist_15* copies resided upstream, 30 in introns, 47 downstream, and three in cds of the differentially expressed genes. Thus, *Tourist_15*s are able to redistribute LHY binding sites upon mobilization. Fifteen of the 28 PCR-assayed *Tourist_15* insertion sites were polymorphic. Thus, they might dynamically rewire the circadian clock network and possibly also accelerate adaptation of carrot to abiotic stresses.

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Keywords: Daucus carota, abiotic stress, gene expression, miniature inverted repeat transposable elements, PIF, Harbinger

*Speaker

Genomic landscape of transposable elements and structural variation in wild house mouse populations across diverse environments

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House mice (*Mus musculus domesticus*) were introduced to the Americas by European colonizers and are now widely distributed from Tierra del Fuego to Alaska. Past studies of wild-caught mice across latitudinal and temperature gradients provided evidence of environmental adaptation in traits related to body size, body proportions, and metabolism. Moreover, genome-wide scans identified candidate genes underlying adaptive differences. However, the contribution of transposable elements (TEs) to environmental adaptation remains unknown in wild populations of house mice. Here, we used PacBio long-read whole-genome sequencing toinvestigate the genomic distribution and variation of TEs, including their classification, distribution, and abundance in wild populations inhabiting contrasting temperate and tropical environments (New Hampshire-Vermont, USA, and Manaus, Brazil). We sequenced 20 individuals to a minimum 25-fold coverage, generating genome assemblies for each individual (N50=20-54 Mb). We identified and classified TEs using RepeatModeler and MCHelper. We manually curated TE library call-sets for both populations, improving TE identification and resolving unknow classifications. Across both wild populations, *LINE/L1* and *LTR/ERV* superfamilies were the most frequently identified TEs. Moreover, we observed that TEs occupy approximately 30% of the genome, with LINE 1 being the most abundant (_˜14% of the genome). Additionally, we identified over 92,000 structural variants (SVs) across all 20 genomes. Compared to mouse inbred strains and human populations, we find 1.8x and 4.5x more SVs, respectively. Our future directions will involve investigating the contribution of structural variation and TE polymorphism to environmental adaptation in natural populations of house mice.

Keywords: house mouse, environmental adaptation, structural variants

*Speaker

Transposable Elements in the UCSC Genome Browser

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The display of transposable elements and their systematic annotation across the thousands of new genomes is a neglected topic in the genomic database ecosystem. The Repeatmasker team and the UCSC Genome Browser group have been working to remedy this.
1) The Browser has a special display mode for RepeatMasker output, available on the human genome, that shows the size of the Repeatmasker query sequence and how the individual pieces of e.g. L1 elements were joined together.

2) Most of the thousands of NCBI assemblies added to the UCSC GenArk collection of genome browsers at https://hgdownload.soe.ucsc.edu/hubs/ were processed with both repeatmodeler and Repeatmasker, and both annotations are available for download and analysis. All VGP (Vertebrate Genome Project) assemblies have been processed like this and a selected set of other assemblies and we can run repeatmodeler on any other set of assemblies on request.

3) The UCSC Repeatbrowser https://repeatbrowser.ucsc.edu/ can show a consensus sequences for each repeat type in the human reference genome and projects various annotations (genes, ENCODE, Chip-Seq) to these consensus sequences.
We present these tools in the hope that they help make transposable elements acessible to a larger audience. We also are looking for feedback on the annotation and display of TE annotations on the Genome Browser.

Keywords: visualisation, genome, genomics, browser, databases

*Speaker

Role of transposable elements in a parasitoid wasp ongoing speciation

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Endoparasitoid wasps lay their eggs and develop at larval stages inside a specific host. Recent studies have shown that parasitoid wasp species often constitute a complex of sister species, each one resulting from the adaptation to a specific host. The project aims to understand the involvement of transposable elements (TEs) in the ongoing speciation of two populations of the wasp *Cotesia congregata* (CcC and MsT) specialized on different hosts. The CcC population parasitizes the caterpillar *Ceratomia catalpa* which lives on the catalpa tree, and MsT population parasitizes the caterpillar *Manduca sexta* which lives on the tobacco plant. Differences in reproductive behavior and genetic differentiation (microsatellites and COI) indicate these two populations are indeed at the beginning of a speciation process. A reproductive defect is observed which could contribute to reinforcing barriers between the two populations. The cross between CcC female and MsT male gives a fertile offspring while the reciprocal cross (MsT female X CcC male) gives a nearly sterile offspring showing ovaries atrophy. We hypothesize that this phenotype corresponds to hybrid dysgenesis previously described in *Drosophila*, which would be induced by a transposable element present in CcC and not in MsT wasps or more global deregulation of TE control. Here, we present results identifying TE candidates potentially involved in dysgenesis by TE comparison from CcC and MsT and the study of the piRNA repression system in somatic and germinal tissues targeting these TEs in both populations using bioinformatic analyses. Among TE candidates are those showing piRNA repression in CcC and not in MsT.

Keywords: hybrid dysgenesis, piRNA, parasitoid wasp, bioinformatics

*Speaker

HERVH protects the human genome from transposition of young retroelements in the early embryo

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The ancestor of human endogenous retrovirus H (HERVH) was among the plethora of retroviruses, invaded primate genomes. HERVH integrated into the primate germline after the divergence of New- and Old-World monkeys (_˜40 MYA). As a result of successful endogenization and transposition, HERVH is represented by about 1,200 genomic copies in the human genome. Although HERVH no longer transposes, several HERVH-containing genomic loci are transcribed in the early human embryo. Indeed, HERVH is an example of co-option by the host. HERVH-derived transcripts form a transcriptional regulatory network that supports pluripotency in humans. HERVH-supported functions (e.g. pluripotency) add primate-specific features to evolutionarily conserved biological. In our recent study, we observed increased retrotransposition of LINE-1 and the occurrence of *de novo* integration of non-autonomous Alu and SVA elements in HERVH-depleted hESCs, suggesting that HERVH is involved in the protection of genome stability. We have shown that a copy inserted upstream of APOBEC3G inhibits retrotransposition of evolutionarily young (< 7MY) retrotransposable elements (REs). Interestingly, HERVH appears to protect genome stability in more than one way. Here, we report a subcluster of HERVH copies (_˜300; HERVH-lin) that has an expression profile opposite to that of young REs and exhibits a tandemly repeated binding motif for the LIN28A protein. LIN28A is an RNA-binding protein, and inhibits the maturation of the miRNA Let-7. The "binary dance" of LIN28A and Let-7 is an evolutionarily conserved regulatory pathway that and establishes a delicate balance between pluripotency and differentiation. Let-7 targets multiple mRNAs, including those encoding pluripotency factors, but also inhibits retrotransposition of LINE-1. Thus, HERVH-lin promotes Let-7-based suppression of LINE-1 retrotransposition (and other non-autonomous retroelements such as Alus and SVAs). Our data suggest that HERVH-lin recruits an evolutionarily conserved regulatory pathway via sponging on LIN28A and integrates Let-7 into the genome protection programme of human pluripotent stem cells.

Keywords: endogenous retrovirus HERVH genome stability LINE, 1 LIN28A Let, 7

*Speaker

The importance of transposable element curation

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Transposable elements (TEs) are drivers of evolution, acting as the substrate by which massive genotypic change can occur. Although often associated with deleterious effects such as disease states, TEs can also provide plasticity to endogenous regulatory networks by contributing additional promoter sequences, protein-coding regions and transcription factor binding sites. In addition, each species contains a unique repertoire of TEs, and will therefore differentially contribute to diversity therein. As such, it is difficult to overstate the importance of careful TE annotation in genome assemblies. With the advent of long-read sequencing technologies at lower cost, it is often tempting to solely rely on high-throughput computational pipelines. While an excellent starting point, automatic pipelines have their drawbacks. For example, novel repeats, such as chimeric elements and undiscovered repeat orders, will likely be overlooked. It is therefore crucial to assess individual models via manual curation in order to produce a high-quality repeat library. For example, a curated library for the human genome reveals an additional 11% TE content over an un-curated RepeatModeler2 library, while an additional 3% can be gained for *Drosophila melanogaster*. Current TE curation practices emphasize the use of consensus sequences. However, by only focusing on the average sequence, the rich information the contributing insertions provide is often ignored or discarded. The full alignment for each potential TE family can provide subfamily information, precise classification, and indication of whether the entire consensus has been discovered. Only with the combination of a high-quality TE library and complete genome assembly can a full picture of evolution take place.

Keywords: curation, multiple sequence alignment, annotation, classification

*Speaker

Dynamic genomes of Hydra reveal anciently active repetitive elements of animal chromosomes

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Animal genomes are characterized by highly conserved chromosomal homologies that predate the ancient origin of this clade. Despite such deep conservation, the mechanisms behind the retention, expansion, and contraction of chromosomal elements and the long-term (macro-evolutionary) functional implication of these processes remain to be elucidated. Here we present a comprehensive stem-cell resolved genomic and transcriptomic study of the fresh-water cnidarian *Hydra vulgaris*, an animal characterized by its high regenerative ability, the capacity to propagate clonally, and an apparent lack of senescence. Utilizing newly generated single haplotype telomere-to-telomere genome assemblies of the two recently diverged hydra strains, we show how the macro-evolutionary history of its chromosomal elements are shaped by ancient and recent transposable element (TE) expansions, which, combined with the distinct strain-specific preference for either sexual or asexual reproduction, is forming divergent evolutionary trajectories in these genomes. By comparing the individual genomes of hydra's three types of stem cell lineages, we show that distinct TE families are actively and preferentially inserting in the genomes of each of the lineages. In whole transcriptomes, over 14,000 transcripts were composed of nearly complete TE sequences, and finer classification into families, subfamilies, and individual loci reveals an increased detection of cell type-specific expressions of TEs. The active TEs include elements that differentially contribute to the changes in the genome size as well as persistent structural variants around loci associated with cell population proliferation. Our study reveals a core set of 14 TE families including 11 DNA elements, 2 LINE elements, and one LTR element that act in this role. The evolutionary analysis of these elements suggests an ancient role in maintaining the evolutionary topology of animal chromosomes.

Keywords: Hydra, Genome expansion, Haplotype resolved telomere to telomere genome assemblies, Stem cell genomes, Anciently active transposable elements

*Speaker

Environment impacts the distribution of Copia elements in metazoan.

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Studying transposable elements in lesser-explored branches of life is essential to understand their distribution and evolutionary dynamics. Over the past years, our investigation into the diversity of LTR-retrotransposons in crustaceans, mollusks, and annelids has enabled us to establish the classification of LTR-retrotransposons into several clades per superfamily, while also revealing strong differences in their distribution. Copia elements appear rare and relatively homogenous in these three taxa, whereas Gypsy elements are detected in a considerable number of copies and exhibit high diversity. BEL/Pao elements show an intermediate behavior.
To complete our exploration of LTR-retrotransposons spread in metazoans, we characterized them in 263 well-assembled genomes covering most of this realm. Based on the phylogeny of RT/RNaseH domain, we annotated each superfamily of elements to the clade level. Our observations revealed differences between taxa. For instance, no Copia or BEL/Pao elements were detected in turtles' genomes, while serpents exhibited a particularly high number of elements. Although Gypsy elements analysis is ongoing, we have already identified several new clades in Copia and BEL/Pao and characterized the distribution of existing clades in metazoans. These clades exhibit various distributions, and surprisingly, our results indicate a strong influence of environment (marine vs terrestrial) on the Copia clade repartition. This suggests a role of horizontal transfers in the evolutionary dynamics of these elements.

Keywords: LTR, retrotransposons, Metazoa, Environement, Evolutionary dynamics

*Speaker

Contribution of variable TE content on phenotype and plasticity in Drosophila melanogaster

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Phenotypic plasticity is the ability for one genotype to yield different phenotypes in response to environmental changes. This process may provide organisms with the capacity to adjust their phenotype to better fit their environment as it changes. Most of the mechanisms that are at play in generating phenotypic plasticity are epigenetic mechanisms, such as histone marks, DNA methylation or small RNAs, allowing differential expression of genes, and eventually result in phenotypic changes. Epigenetic mechanisms have often been documented to fluctuate with the environment. Not only these mechanisms regulate gene expression, but they are also involved in the repression of transposable elements (TEs). TEs are repeated DNA sequences potentially capable of moving within genomes. Thanks to their transposition mechanisms, but also to their inherent regulatory sequences, TEs are known to impact gene expression, genome architecture and integrity. We hypothesize that TEs' control mechanisms and regulatory components, might also vary with the environment and potentially lead to changes in TE expression and transposition rate, and in turn alter gene expression. To test this hypothesis, we investigated the influence of TE content on phenotypes in Drosophila melanogaster, and as a potential driver of phenotypic plasticity. We took advantage of genetically engineered fruit flies that bear different TE content but identical genetic background, to tackle this question in a controlled manner. First, phenotypic tests were conducted and revealed significant differences between the lines harbouring different TE content. We further investigated the plastic response of these phenotypes in a variety of environments and stressors. Results depict genotype by environment interactions for multiple traits measured in variable environments, suggesting that indeed TEs contribute to plasticity.

Keywords: plasticity, transposable elements, piRNA, Drosophila

*Speaker

Evolution of Drosophila paralogs in the light of their transposable element neighborhood

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Gene duplication is an evolutionary mechanism providing new genetic material and opportunities to acquire new functions. After duplication, paralogs can encounter various fates. A large number is lost via pseudogenization due to the accumulation of deleterious mutations. However, a significant proportion is maintained through different paths (neo-functionalization, sub-functionalization and function redundancy).
To investigate how duplicated genes are maintained in the Drosophila melanogaster genome, we explored the function over-representation of all paralogs compared to singleton genes. We also considered the transposable element (TE) environment of genes. TEs are repeated sequences that can influence the expression of genes in their vicinity. They could thus influence the fate of duplicated genes. Our results show that both duplication status and the TE environment are associated with functional biases.

We also determined the expression divergence of pairs of duplicated genes and observed that it is associated to different factors like the age to duplication, the gene pairs localization and the TE neighborhood. In particular, the gene expression is more conserved when TEs are present in the vicinity of duplicated pair of genes compared to gene pairs devoid of TEs.
Our results allow to hypothesize that the presence of TEs near duplicated genes may be associated to their evolutionary fate.

Keywords: gene duplication, transposable element influence

*Speaker

A role for transposons in the evolution of programmed DNA elimination in Mesorhabditis nematodes

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While we commonly assume that individual organisms carry an identical genome across cells and tissues, a number of eukaryotic species undergo Programmed DNA Elimination (PDE), the destruction of parts of chromosomes in somatic cells during normal development. How and why PDE occurs has remained largely unresolved, largely due to a lack of lab-tractable models and insufficiently complete sequencing data (e.g., high-fidelity long-reads).
We recently discovered that species of Mesorhabditis, a genus of lab-tractable nematode worms, undergo substantial PDE during early development (_˜30% of the genome is eliminated). In this talk, I will first introduce how we are probing the PDE process in Mesorhabditis using both experimental and computational approaches. I will then focus on how, using a combination of PacBio Hi-Fi, Illumina and Hi-C data, we could resolve elimination breakpoints and identify a clear sequence motif specifying genome cutting. Surprisingly, we then found that a majority of these motifs are located inside transposable elements (TEs), and that some breakpoint motifs have moved recently under TE control.
I will end by discussing our ongoing search for the effector nuclease(s) of PDE and whether it is TE-encoded, and the evolution of PDE including, ultimately, whether PDE benefits the host or is a TE-encoded 'selfish' process.

Keywords: Programmed DNA Elimination, Genome Evolution, Transposable elements, Nucleases, Genome cutting, Soma/Germline Differentiation, Development, Nematodes

*Speaker

Systematic annotation of Helitron-like elements in eukaryote genomes using HELIANO

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Helitron-like elements (HLEs) are widespread eukaryotic DNA transposons employing a rolling-circle transposition mechanism. Despite their prevalence in fungi, animals, and plant genomes, identifying *Helitrons* remains challenging. We introduce HELIANO, a software for annotating and classifying autonomous and non-autonomous *Helitron* and *Helentron* sequences from whole genomes. HELIANO outperforms existing tools in speed and accuracy, demonstrated through benchmarking and its application to complex genomes (*Xenopus tropicalis, Xenopus laevis, Oryza sativa*), revealing numerous newly identified *Helitrons* and *Helentrons*. In a comprehensive analysis of 404 eukaryote genomes, we found HLEs widely distributed across phyla, with exceptions in specific taxa. *Helentrons* were identified in numerous land plant species, and 20 protein domains were discovered integrated within specific autonomous HLE families. A global phylogenetic analysis confirmed the classification into main clades *Helentron* and *Helitron*, revealing nine subgroups, some enriched in particular taxa. The future use of HELIANO will contribute to the global analysis of TEs across genomes and enhance our understanding of this transposon superfamily. Please check the full article from the following link: https://www.biorxiv.org/content/10.1101/2024.02.08.579435v1.

Keywords: Helitron, prediction, Phylogegetic, gene capture

*Speaker

Coevolution between transposable elements and fungal plant pathogen genomes shapes the genome architecture, plasticity, and adaptation

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Since the initial discovery of transposable elements (TEs), growing evidence supports their impact on genome evolution, sometimes acting as a driving force for rapid adaptation. Fungi provide unique systems for studying TE-host genome coevolution due to their wide variation in TE coverage, linked to diverse fungal lifestyles, demographic events, and mechanisms regulating transposition. TE activity fosters genetic variability of fungal pathogens directly through insertion within coding or regulatory regions and indirectly by inducing epigenetic modifications. Notably, TE-driven modifications can significantly affect fungal pathogenicity by targeting genes involved in virulence. Our research aims to unravel how the coevolution between transposable elements and host genomes has shaped genome evolution, architecture, and adaptation in fungal pathogens. We concentrate on two extremes: firstly, rust fungi, obligate biotrophic fungal plant pathogens characterized by remarkably large genomes (80Mb-1Gb) with high TE coverage (50-90%); secondly, the hemibiotroph *Zymoseptoria* species-complex, featuring smaller (40Mb), compartmentalized genomes with lower TE coverage (10-25%). Our approach combines the comprehensive characterization of complete TE repertoires in high-quality reference genomes with transcriptomics, epigenomics, and experimental evolution to explore the dynamics between TE mobility and host-genome regulation mechanisms. Our analyses unveil that genome expansions in rust fungi primarily result from retrotransposon bursts, punctually occurring throughout the evolutionary history of rust fungi. In the *Zymoseptoria* species-complex, variation in TE mobility correlates with the recent loss of DNA methyltransferase activity and a reduction in Repeat-Induced point mutation (RIP-like) signatures during mitotic proliferation. In shedding light on the different TE dynamics in various fungal pathogen species, we provide new insights into how the different levels of genomic tolerance against TEs reshape eukaryotic genomic landscapes.

Keywords: fungal transposable elements, plant pathogens, genome evolution, repeat induced point mutations, epigenomics

*Speaker

Deciphering the role of miR845 in the epigenetic regulation of transposable elements in Brassicaceae

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In plants, transposable elements (TEs) are predicted targets of endogenous microRNAs (miRNAs), suggesting that miRNA-TE interactions could represent an ancient evolutionary force driving epigenetic regulation and genome expansion. This includes the highly conserved miR845 family that targets specifically retrotransposons in *Arabidopsis* pollen, in a mechanism that has been implicated in the regulation of parental genome dosage in triploid seeds (1). However, the role of miR845 in epigenome reprogramming and TE evolution remains poorly understood. Interestingly, natural variation in miR845 activity was observed in *Arabidopsis thaliana*, where the loss of miR845 correlates with increased TE expression in pollen and low triploid seed abortion (1). In addition, within the Brassicaceae family, we have recently confirmed the absence of functional *MIR845* genes in *Arabis alpina*, where a massive genome expansion resulted from increased activity of predicted miR845 targets (2). In order to investigate the role of miR845 in these species, we have generated *MIR845* complementation lines of *A. thaliana* Ler-0 and *A. alpina*, which were able to restore strong miR845 expression in pollen. I will present our preliminary small RNA and transcriptome analysis of these lines, where we expect to find important mechanistic insight on the role of miR845 in the epigenetic control of TEs during reproduction and seed development. Ultimately, we expect that this work will allow to elucidate the impact of miR845 in the evolution and expansion of retrotransposons in plant genomes.

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Keywords: microRNAs, miR845, Retrotransposons, Brassicaceae, Epigenetic Control, Pollen, Reproduction

*Speaker

Epi/genetic adaptation of perennial plants to climate change, OPTIMAE project

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In the current context, adapting crops to climate change is a major objective for agriculture. For several years, we have been witnessing an acceleration in climate change in Europe and around the world. Crops will have to cope with increasingly frequent episodes of intense heat and drought, leading to water restrictions. Varieties planted in orchards today will have to cope with conditions they have never faced before, and which will potentially have major consequences, even their survival. Very few genes involved in tolerance to abiotic stresses are known in perennial species. In addition, it is becoming essential to find alternatives for adapting current varieties to future climatic conditions. Recently, Transposable Elements (TEs), mobile DNA sequences playing an important role in genome evolution and in regulating the expression of genes located nearby, have been linked to the adaptation of plants to their environment. Our project will use 2 original approaches to study the impact of TEs on apple breeding : 1) To adapt to apple trees a method for accelerating the natural process of TE neoinsertion *via* the combined application of a drug and abiotic stress. Epi/genetic modifications generated by this method would enable us firstly to validate the primordial role of TEs in the adaptation of this species to climate change, and secondly to obtain genotypes potentially better adapted to the applied stress and 2) To study the ongoing natural epi/genetic adaptation of Golden Delicious, a model of apple tree variety, adapted for decades to extreme and contrasting environments. These two approaches will then enable us to identify "marker" genes or alleles whose altered expression levels lead to increased tolerance to applied abiotic stresses. These data can then be tested in Marker-Assisted Selection in collaboration with breeders to obtain varieties that are better adapted to their environments.

*Speaker

Keywords: Adaptation, climate change, epigenetics, Transposable Elements (TE), Malus domestica

LTR-checker: deep-learning (DL) guided structural identification of LTR retrotransposon providing decreased computation demand and high flexibility

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- LTR retrotransposons (LTR-RTs), ubiquitous and dominant component in plant genomes, are playing critical roles in functional variation, genome plasticity and evolution. While the computational demand is still high for LTR-RT identification, especially for large genomes such as wheats (5-15 Gb) and conifers (10-40 Gb). Significant accumulation of whole-genome sequences following the ever-advancing sequencing technologies is calling for efficient computational tools in LTR-RT identification.
- Here, we generated a deep-learning (DL) guided LTR-RT identification method, LTR-checker, which provides decreased computation demand and high flexibility. (1) We collected full-length LTR-RTs from most released plant whole genomes reflecting broad phylogenetic representativeness, thus captured the broad LTR-RT diversity, and constructed a consensus LTR-RTs dataset. (2) With the consensus LTR-RT dataset, a DL model of convolutional neural network (CNN) was built to predict the occurrent of LTR-RT. And a new method was created by using DL model, as a guider, to direct the finer structural identification procedure to potential LTR-RT location in the whole genomes, so as to decrease the computational demand. (3) We examined the performance of LTR-checker by comparing it with poplar LTR-RT identification methods. A dataset composed of 200,000 consensus LTR-RTs was generated, and publicly available at: https://zenodo.org/records/10454902. Our DL model shows very promising accuracy in LTR-RT prediction. Our new method, LTR-checker, shows competitive performance with poplar methods (LTR-finder, LTR-harvest, LTR-retriever), and it achieves 10-20x faster in CPU-time and for large genomes (maize, wheat, pine), and 4-5x lower in computer RAM memory. The low computational demands enable high flexibility in LTR identification by detecting hundreds of nested LTR-RTs when loosening the range width settings in genomic survey of LTR-RT. The new method is publicly available at: https://github.com/morningsun77/ltr_checker.

Keywords: LTR retrotransposons (LTR, RTs), Deep learning, Structural identification, Large genomes

*Speaker

Manual versus automatic annotation of TEs: case studies from Drosophila melanogaster and Aedes albopictus

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The identification of transposable elements (TEs) in sequenced genomes remains a challenge. While pipelines like RepeatModeler2, REPET, and EDTA have been developed to detect repeated loci within genomes by comparing them with curated TE sequences, automated detection algorithms often lack the precision needed for certain applications. This calls for manual curation, which can be time-consuming and challenging for non-specialists but serves as a validation for computational predictions and allows researchers to refine results based on specific biological contexts. Tools for automated curation, such as EarlGrey and MCHelper, show promise in improving the quality of TE libraries and aiding manual curators. However, researchers face a dilemma between automated and manual approaches, potentially impacting the final library and downstream analyses. To compare these approaches, we assessed the efficiency and accuracy of a manual curation pipeline versus an automatic one.

We benchmarked the two approaches on the genomes of Drosophila melanogaster and Aedes albopictus. *D. melanogaster*, extensively studied, already has a comprehensive and high-quality TE characterization. In contrast, Ae. albopictus is a non-model species with a large genome (10 times that of *Drosophila*) and a poorly characterized TE landscape. For each genome we obtain two libraries which mostly do not overlap in terms of sequence identity. Contrasting between manual and automatic curation pipelines, the most striking difference is the fragmentation of automatic curation, especially for LTR elements. As expected, in D. melanogaster we found the genomic coverage to be similar between the two approaches, while a much marked discrepancy emerged in Ae. albopictus, for which the manual curation was known to be non-exhaustive. Our results highlight differences in the outcome of manual versus automatic TEs curation processes, suggesting their complementarity in order to obtain an exhaustive and accurate TE library. Finally, this analysis provides a unified high quality library for Ae. albopictus.

*Speaker

Keywords: Transposable elements annotation, BIoinformatics, Benchmark

Effective population size does not explain long-term variation in genome size and transposable elements content variation

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Animal genomes exhibit a remarkable variation in size, but the evolutionary forces responsible for such variation are still debated. As the effective population size (Ne) reflects the intensity of genetic drift, it is expected to be a key determinant of the fixation rate of nearly-neutral mutations. Accordingly, the Mutational Hazard Hypothesis postulates lineages with low Ne to have bigger genome sizes due to the accumulation of slightly deleterious transposable elements (TEs), and those with high Ne to maintain streamlined genomes as a consequence of a more effective selection against TEs. However, the existence of both empirical confirmation and refutation using different methods and different scales precludes its general validation. Using high-quality public data, we estimated genome size, TE content and rate of non-synonymous to synonymous substitutions (dN/dS) as Ne proxy for 807 species including ray-finned fishes, birds, mammals, molluscs and insects. After collecting available life-history traits, we tested the associations among population size proxies, TE content and genome size, while accounting for phylogenetic non-independence. Our results confirm TEs as major drivers of genome size variation, and endorse life-history traits and dN/dS as reliable proxies for Ne. However, we do not find any evidence for increased drift to result in an accumulation of TEs across animals. Within more closely related clades, only a few isolated and weak associations emerge in fishes and birds. Our analyses outline a scenario where TE dynamics vary according to lineage-specific patterns, lending no support for genetic drift as the predominant force driving long-term genome size evolution in animals.

*Speaker

Keywords: Genome size, Transposable elements, Mutational hazard hypothesis, Genetic drift

The adaptive and variable transposable elements landscape of mosquitoes (Culicidae)

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Mosquitoes (family Culicidae) are among the most relevant species for human health, being the primary vectors for pathogenic viruses, nematodes, and protozoa. Several members of the family have become invasive in Europe due to human-mediated introductions from their typically subtropical native ranges. Despite the often severe bottlenecks associated with the invasive process, these species, such as Aedes albopictus in Italy, demonstrate a remarkable ability to rapidly adapt to a wide range of environmental conditions. This phenomenon, often referred to as the "genetic paradox of invasive species," has been previously observed in other organisms, with transposable elements (TEs) hypothesized to play a primary role as mutational agents, allowing such rapid adaptations. Here, we present a multidisciplinary approach aimed at elucidating this process by: (a) studying the TE landscape across the entire Culicidae family and, on a population level scale; (b) investigating the role of transposons in the adaptive potential and, therefore, invasiveness of A. albopictus across the Italian peninsula. We are currently compiling a comprehensive dataset of whole genome sequencing from publicly available resources covering most of the Culicidae diversity. This dataset will be used to estimate the TE content and its evolutionary dynamics through the clade using a reads-based approach. At the same time, we are setting up an experimental design including lab-reared and natural populations sampled along temperature gradients across Italy to test the hypothesis that TEs are mobilized under (thermal) stress. Overall, these complementary analyses have the potential to provide novel insights into mosquito genome evolution and invasiveness.

Keywords: Mosquitoes, adaptation, invasive species, thermal stress

*Speaker

Identifying LTR-retrotransposons inserted as tandem elements sharing internal LTRs

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Transposable elements (TEs) can mobilize inside host genomes, potentially creating genetic variability. Long terminal repeat (LTR) retrotransposons (LTR-RTs) are the most prevalent transposable elements in plants species. Here we show that the analysis of an LTR-retrotransposon insertion in rice linked to an agronomic trait revealed a complex structure with two LTR-RTs tandemly repeated and sharing the internal LTR. This structure is flanked by Target Site Duplications (TSDs) suggesting that it may be the result of a single insertion. The analysis of the genomes of 81 rice varieties assembled from long-read sequences showed that this region is highly polymorphic, with some varieties harboring the entire LTR-RT tandem while others contained the insertion of a single LTR-RT. In order to study the prevalence of these structures we designed IDENTAM, a tool for identifying LTR-RT tandems sharing internal LTRs. Running IDENTAM on the *Nipponbare* rice accession led us to find 35 putative LTR-RT tandem insertions. In most cases the tandems are flanked by the TSDs and show high similarity between LTRs and between the internal regions, supporting the recent insertion of these structures. Our preliminary analysis of other plant genomes suggest that this is a common phenomenon, at least in plant genomes, that has been overlooked until now.

Keywords: LTR, retrotransposons, rice, tandem insertions

*Speaker

Evolutionary Dynamics, and Functional Implications of the Antiviral Immune Protein Kinase R (PKR) Locus in Myotis Bats

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Gene copy number variation (CNV) participates in shaping innate immunity. CNVs and subsequent gene divergence may be adaptive in host-pathogen evolution. In bats, duplication and positive selection of specific antiviral genes have contributed to bat-specific adaptation against viruses. Here, we studied the Protein Kinase R (PKR), a broad antiviral interferon-induced factor and global translation regulator, in *Myotis* bats to understand the dynamics of its duplication and consequences to innate immunity.

We performed in-depth characterizations of *Myotis* PKR evolution at the genomic, genetic, and transcriptomic levels. We used newly-generated genomes of nine species and transcriptomics from primary fibroblasts of 15 individuals under immune stimulations. Through functional assays, we investigated paralog co-expression in antiviral restriction and translation shutdown.

We found significant variation within the PKR locus, with at least three genomic losses since the original PKR duplication. We also found enrichment of TEs in this locus and qualitative TE differences between paralogs, suggesting TEs may participate in its genomic plasticity.
Furthermore, at basal and upon stimulation by pathogen-associated molecules or interferon, transcriptomics revealed an apparent bias in endogenous gene expression between paralogs. We hypothesize that variability in expression could be attributed to distinct regulatory elements, potentially influenced by the different TE landscapes.
In exogenous assays, we further found intrinsic differences in paralog protein steady-state expression levels. Despite one copy being poorly expressed, our functional assays revealed its strong activity in shutting down protein translation and restricting viral infection. We are currently assessing whether the copies may regulate one another and perform additive functions.

The evolutionary dynamics of the *Myotis* PKR locus, with possible implications of TE contribution, may be the result of lineage-specific selective pressures, potentially driven by viral

*Speaker

epidemics and costs of PKR duplication. Overall, our work may contribute to a better understanding of adaptive duplication dynamics in bat innate immunity.

Keywords: bat innate immunity, Protein Kinase R, gene duplication, gene copy number variation

REXdb: Update coming soon

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1

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REXdb is a comprehensive database of conserved protein domain sequences extracted from all TE types occurring in plants (1). It serves as a basis for the automatic classification of TEs identified with the RepeatExplorer pipeline, but can also be used independently with the DANTE (Domain Based Annotation of Transposable Elements) tool (both available at http://repeatexplorer.org/). Recently, we have implemented the database in DANTE-LTR, a new tool for the identification and hierarchical classification of LTR retrotransposons in lineages (see the poster by Petr Novák). Since the current version of REXdb (Viridiplantae_v3.0) mainly covers domains extracted from elements of angiosperm species, the use of the database for gymnosperms and non-seed species is limited due to lower sensitivity and imperfect classification of some elements. Therefore, we set out to perform a comprehensive study of LTR retrotransposons in these species as well. We found that although the sequences of the reverse transcriptase, RNase H and integrase domains were sufficiently conserved to be unambiguously identified, their divergence from the sequences in REXdb often led to incomplete or even incorrect classification. This was even more dramatic for the GAG and protease domains, which showed a very high sequence divergence that in some cases prevented their reliable identification. Phylogenetic analysis revealed that although some lineages of LTR retrotransposons are common to angiosperms, gymnosperms and non-seed plants, the non-seed plants also contain elements constituting lineages that are missing in the current REXdb classification system. These results have prompted us to expand REXdb to 1) include additional sequences of non-angiosperm elements and 2) incorporate new findings in the phylogeny of plant LTR retrotransposons into the associated classification system. To make our data available to the community, we will soon release a new version of REXdb, Viridiplantae_v4.0. (1) Neumann et al. 2019, *Mobile DNA* **10**, 1. DOI: 10.1186/s13100-018-0144-1

Keywords: REXdb, LTR retrotransposons, protein domain, plants, Viridiplantae

*Speaker

DANTE and DANTE_LTR: computational pipelines implementing lineage-centered annotation of LTR-retrotransposons in plant genomes

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Long terminal repeat retrotransposons (LTR-RTs) significantly contribute to the genomic composition and evolution of higher plants, comprising up to 75% of their nuclear DNA. These elements have roles that range from causing genomic disruption to facilitating adaptive changes. Traditional methods for the identification and annotation of LTR-RTs have primarily relied on structure-based approaches, focusing on LTR sequences and generally limiting classification to the Ty3/gypsy and Ty1/copia superfamilies. However, these methods face challenges in genomes with high levels of tandem repeats or nested LTR-RT insertions, and often result in a significant number of false positives.

To improve upon these limitations, we introduce DANTE, a computational pipeline for Domain-based Annotation of Transposable Elements that performs classification of the elements into phylogenetic lineages based on sequences of their conserved protein domains. The identified protein domains are then used by the DANTE_LTR pipeline to annotate complete element sequences by searching for their structural features, such as long terminal repeats, in the adjacent genome regions. As a reference protein database, DANTE and DANTE_LTR use the REXdb database, which contains over 75,000 conserved protein domains from all types of transposable elements found in plants. This protein domain-centered approach enables the subsequent identification of LTR-RT structural features with greater accuracy and a reduction in false discoveries. Moreover, the approach allows for the classification of elements at a more detailed lineage level rather than limiting to broad superfamilies, providing deeper insights into their diversity and evolutionary history.

The DANTE and DANTE_LTR tools are available in command-line versions and also from the Galaxy interface on our RepeatExplorer server (https://repeatexplorer-elixir.cerit-sc.cz/).

References:

Neumann, P., Novak, P., Hostakova, N., Macas, J. (2019) – Systematic survey of plant LTR-retrotransposons elucidates phylogenetic relationships of their polyprotein domains and provides a reference for element classification. Mobile DNA 10:1.

Keywords: REXdb, LTR retrotransposons, protein domain, plants, Viridiplantae, annotation

*Speaker

MCHelper automatically curates transposable element libraries across species

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The number of species with high quality genome sequences continues to increase, in part due to scaling up of multiple large scale biodiversity sequencing projects. While the need to annotate genic sequences in these genomes is widely acknowledged, the parallel need to annotate transposable element sequences that have been shown to alter genome architecture, rewire gene regulatory networks, and contribute to the evolution of host traits is becoming ever more evident. However, accurate genome-wide annotation of transposable element sequences is still technically challenging. Several de novo transposable element identification tools are now available, but manual curation of the libraries produced by these tools is needed to generate high quality genome annotations. Manual curation is time-consuming, and thus impractical for large-scale genomic studies, and lacks reproducibility. In this work, we present the Manual Curator Helper tool MCHelper, which automates the TE library curation process. By leveraging MCHelper's fully automated mode with the outputs from two de novo transposable element identification tools, RepeatModeler2 and REPET, in fruit fly, rice, and zebrafish, we show a substantial improvement in the quality of the transposable element libraries and genome annotations. MCHelper libraries are less redundant, with up to 54% reduction in the number of consensus sequences, have up to 11.4% fewer false positive sequences, and also have up to ∼45% fewer "unclassified/unknown" transposable element consensus sequences. Genome-wide transposable element annotations were also improved, including larger unfragmented insertions. Currently MCHelper includes a module to perform manual inspection of consensus sequences. However, to fully automate the curation process, we designed a post-processing workflow based on deep learning to automate the inspection of the consensus sequences and generate a ready-to-use curated library. MCHelper is a fast, easy to install, and easy to use tool and is available at https://github.com/GonzalezLab/MCHelper.

Keywords: automatic curation, annotation, MCHelper, bioinformatics, tool, deep learning

*Speaker

Mechanism of invasion and transposition of somatic transposable elements

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Long Terminal Repeat (LTR) retrotransposon represent a class of transposable elements (TEs) found in nearly all eukaryotic genomes. In Drosophila melanogaster, 11% of the genome is made up of LTR retrotransposons and their remnants. TEs can persist in a species only if they are capable of transposing into the germline lineage. Interestingly, errantiviruses, a subclass of LTR retrotransposons, which share structural and functional similarities with vertebrate endogenous retroviruses (ERV), persist in the Drosophila genome despite being expressed exclusively in somatic cells of the ovary. The transposition cycle of errantivirus and the mechanisms that enable them to transit from somatic cells to germ cells, and thus invade the genome of subsequent generations, remain enigmatic. To elucidate these mechanisms, this study will focus on a specific Drosophila errantivirus, *ZAM*. We created conditions in which the control of *ZAM* was abolished leading to its *de novo* reactivation in somatic gonadal cells and subsequent invasion of the germline (Yoth et al., 2023). Using this model and a combination of genetic, biochemical, and imaging approaches, my PhD project aims to dissect the molecular mechanisms involved in ERV infection and transposition.

Keywords: Errantivirus (Endogenous retrovirus, like), Invasion, Transposition, env

*Speaker

The role of transposable elements in the evolution of reproductive barriers in hybridising birds

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The molecular determinants underlying reproductive barriers between species can be very different from species to species. Recently, structural variants (SVs) are getting more and more attention as facilitators of the evolution of genetic incompatibilities and eventually of reproductive barriers. Inversions are considered particularly important in this context as they can easily link multiple incompatibility loci at once and then reinforce postzygotic barriers. In general, apart from inversions, SVs and notably transposable elements (TEs) are an underestimated source of incompatibilities.
Here, we aim to investigate how hybridisation itself can trigger the generation of new SVs by causing a partially uncontrolled TE activity in hybrids. TEs are known to increase in activity in various hybrid organisms and we hypothesize that the uncoupling of active TEs and their repressors caused by hybridisation and recombination can escalate genetic conflicts in two ways. First, increased TE activity can cause more insertions (SVs per se) and the origin of new incompatibility loci. Second, new homogenous insertions can act as substrate for further SVs through non-allelic homologous recombination between these repeats (e.g., inversions). This hypothesis predicts SVs and TEs to be a preferential source of incompatibilities, then we should be able to see that regions resilient to gene flow between the hybridising species are denser in SVs and that SVs are preferentially induced by TEs.
We test this hypothesis using an extensive phased genomic dataset of 300 wheatears from the *hispanica* species complex that entails hybridization in multiple contact zones between different combinations of species.

Keywords: hybridisation, secondary contact, structural variants, genetic conflict, gene flow, birds

*Speaker

soloLTRseeker: a new tool to identify soloLTR sequences

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Transposable elements (TEs) are mobile DNA sequences present virtually in all eukaryotic organisms. They are repetitive sequences that can form complex structures, which are problematic to annotate and resolve. Recent advances in sequencing technologies have accelerated the publication of high-quality genome assemblies, often sequenced from telomere to telomere and including regions of high TE density. These developments have brought about a flurry of algorithms and annotation tools that collectively aim to help researchers study TEs instead of masking out their sequences. One type of TE structure, however, remains hard to identify, with scientists resorting to ad hoc approaches for their annotation. These structures, known as soloLTRs, are the product of unequal homologous recombination between the LTRs of the same or closely located LTR retrotransposons. During soloLTR formation, the internal region between both LTRs and one of the two LTRs are deleted, leaving a single LTR. This is a dynamic process that has been observed across species. Quantifying these events, and comparing its intensity and impact between hosts requires accurate and efficient soloLTR annotation. We have therefore developed a computational pipeline, soloLTRseeker, tailored for this task. soloLTRseeker requires as input the fasta and gff3 files of the full-length sequence and LTRs of intact LTR retrotransposons. Through a series of steps and filters, soloLTRseeker annotates high-quality soloLTRs that contain target-site duplications, but also generates multiple intermediate files and analysis. The pipeline incorporates the TEsorter tool to allocate elements of the Ty1/Copia and Ty3 superfamilies into lineages (e.g. ATHILA, SIRE), and is therefore suited for plant genomes only. Although still under development, we ran soloLTRseeker in several angiosperm and gymnosperm species, and our tests show that it generates high-quality annotations. Overall, our aim with soloLTRseeker is to develop a tool specifically designed to identify soloLTRs, and fill this gap in TE annotation pipelines.

Keywords: soloLTR, annotation

*Speaker

A tale of two retrotransposons: Spoink and Shellder, the ultimate invaders

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Spoink and *Shellder*, two LTR retrotransposons, recently jumped into the genomes of *Drosophila melanogaster* and *Drosophila simulans* through horizontal transfer (HT). The likely donor species is a *Drosophila* species from Neotropical America, and the events are dated around 40 years ago. By analysing extensive time series datasets of natural *Drosophila* populations, we precisely determined the timing of these HTs and used phylogenetic methods to infer the direction of the HTs.
These initial HT events triggered a cascade of TE invasions in other *Drosophila* species globally, originating from the cosmopolitan species (*D. melanogaster* and *D. simulans*), thus revealing a previously unknown ecological mechanism with profound impacts on genome evolution. Our proposal suggests that human activities are promoting these cascades of HT by facilitating habitat and population expansions of commensal and invasive species. This puts new species into contact, providing more opportunities for transposons to invade.

Keywords: horizontal transfer, retrotransposon, drosophila, evolution, invasive, melanogaster, simulans, ecology, HT

*Speaker

Impact of transposable elements on transcriptional diversity in stressed plants

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Alternative splicing (AS), alternative transcriptional start site (ATSS) using proximal or distal promoters, and alternative transcriptional termination site (ATTS) using alternative polyadenylation (APA) signals are fundamental processes allowing a swift genetic rearrangement of messenger RNA molecules into various RNA isoforms with potentiality for proteome diversity. In plants, modulations of ATSS, ATTS and AS can occur in response to environmental stimuli such as heat stresses, most likely to optimize plant thermotolerance. Accordingly, mutant plants impaired in splicing factors are more sensitive to heat, and AS might be an important component of heat-shock memory.
Transposable elements (TEs) are DNA elements representing significant proportions of plant genomes, usually transcriptionally repressed. Previously seen as invasive junk DNA, there is increasing evidence that TEs contribute to host genetic innovation throughout evolution. Particularly, TEs can colonize genes, and therefore they can be involved in AS, ATSS or ATTS events when those genes are transcribed. In this project, we propose to decipher the contribution of these intragenic TEs in host genetic diversity in two different plant species (*Arabidopsis thaliana* and *Solanum lycopersicum*) subjected to two temperature regimes (20^{o}C and 37^{o}C). To do so, we are using an integrated approach combining long read Oxford Nanopore Technologies (ONT) direct RNA sequencing (ONT-DRS) and Illumina short-read RNA sequencing (shR RNA-seq) that will allow to fully picture RNA isoforms and assess the impact of TEs on genetic innovation by AS, ATSS or ATTS events in response to heat stress.

Keywords: transposable elements, alternative splicing, Direct RNA sequencing

*Speaker

Aphid defense strategies, symbiotic dynamics and the role of transposable elements in parasitoid interactions

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Parasitoids exert strong selective pressure on their hosts, leading to the evolution of defensive traits in aphids to thwart successful parasitism. The interplay of symbiotic associations also influences the evolution of defenses against natural enemies. Parasitism experiments with *Diaeretiella rapae* and *Myzus persicae* aphid isolines, infected or not with the secondary symbiont *Rickettsia*, showed variable parasitism success rates ranging from 43% to 76%. Six *M. persicae* isolines (three with high parasitism rate; three with low parasitism rate) were selected for biological analysis and evaluation of parasitoid behavior during patch exploitation and aphid defensive behavior during parasitoid interactions. Biological parameters revealed remarkable differences among *M. persicae* isolines, which responded differently to parasitism, suggesting potential adaptive costs associated with lower parasitism rates. Furthermore, it was observed that the secondary symbiont *Rickettsia* does not confer additional defense mechanisms to *M. persicae* against *D. rapae*. Behavioral studies revealed differences in the occurrence of host evaluation between low and high parasitism isolines. Specifically, the presence of Rickettsia was found to affect the defensive behavior of *M. persicae* in response to parasitoid attack, thereby interfering with the host selection process of the parasitoid *D. rapae*. Phenotypic differences observed in response to parasitism by *D. rapae* in the *M. persicae* isolines studied, as well as in biological and behavioral assays, could be attributed to genetic variability. We hypothesize that transposable elements (TEs) might be involved as a selective force and contribute to the accumulation of mutations within the host, and potentially assist in host defense responses. Therefore, the aim of our research was to detect and annote TEs in the six different clonal lines, and to verify whether they are associated with genes involved in host immune responses against the presence of macro-invaders, such as parasitoids.

Keywords: Host, parasitoid interactions, Transposable elements, Host adaptability

*Speaker

TETrimmer: a tool to replace and assistant transposable element manual curation

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Transposable elements (TEs) are repetitive DNA elements that can change their position within a genome. They can occupy large proportions of eukaryotic genomes. Many tools have been developed for *de novo* TE identification, like RepeatModeler, EDTA, REPET, HiTE, and EarlGrey. But manual curation is still required for a high-quality TE annotation by experts, which is very time-consuming. We developed a software called TETrimmer that can replace the main tasks of TE manual curation. Because the sequence divergence among TE subfamilies can be small, more than one type of subfamilies was usually included into one file after BLASTn and multiple sequence alignment (MSA). TETrimmer combined maximum likelihood phylogenetic tree and DBSCNA methods to efficiently cluster and separate MSA based on sequence relatedness. Annotated TEs from *de novo* TE annotation software can be fragmented. TETrimmer can automatically identify the proper extension size, clean the MSA, and define TE boundaries. The cleaning module of TETrimmer is very powerful, it uses new algorithm to efficiently remove MSA gaps and low conserved regions. Finally, TETrimmer supplies a graphical user interface to allow the user easily reviewing and modifying TETrimmer outputs. So far, we have tested TETrimmer on *Drosophila melanogaster*, *Danio rerio*, *Oryza sativa*, *Zea mays*, *Blumeria hordei*, and *Homo sapiens*. Comparing with the directly RepeatModeler2 outputs, TETrimmer can dramatically increase the TE annotation quality.

Keywords: manual curation, annotation, transposable element, RepeatModeler, EarlGrey

*Speaker

Exploring the contribution of transposable elements to the evolution of tetrapod developmental and regenerative programs

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More than 360 million years ago during the Devonian period, a group of lobed-finned fishes undertook the water-to-land transition and became the first group of vertebrates to venture into land: the tetrapods. This transition was enabled by a number of evolutionary innovations which include the transformation of the fish fin into the tetrapod limb and the emergence of a series of novel organs and structures such as the tongue, the bone marrow, the urinary bladder or the second atria of the heart. In this work, we explore what were the genome dynamics at play during the birth of these new organs and scrutinize the hypothesis that transposable elements might have driven the evolution of their tissue-specific cis-regulatory elements. To this end, we have generated a new assembly for the giant Axolotl genome (Ambystoma mexicanum, 32 Gbp) and have aimed to comprehensively characterize its repeat content. These analyses have revealed that the Axolotl genome harbors an extremely diverse collection of transposable element families spanning all major classes and that their genome has grown to such a huge scale due to the retention of ancient repeats. We are currently in the process of accurately dating these transposable element insertions and determining whether they played any role in catalyzing the emergence of the major tetrapod evolutionary innovations. Furthermore, salamanders such as the Axolotl are unique among tetrapods in that they possess the fascinating capacity to regenerate nearly every organ throughout their lives. Why is this the only tetrapod taxon harbouring such a striking regenerative potential and whether this holds any relationship with their exceptional TE-content remain outstanding questions in regenerative research.

Keywords: evolution, tetrapods, regeneration

*Speaker

A physiological explanation for the abundance of transposon DNA in bacteria

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Transposon DNA is highly abundant in genomes, even in microbes. Typically, 1-30% of microbial genomes are active transposons. Surprisingly, the biological role of transposons within microbial genomes remains poorly understood. Transposons are disrupters of genetic information, involved in pseudogenization, the deterioration of genomes and key to the breakdown of product expression in biotechnology. On the other hand, transposons contribute to genetic change and adaptation by their importance for genetic recombination and the generation of beneficial mutations. Here, we experimentally test for biological effects of transposons on a whole-genome level – when they are not transposing. We ask whether transposons are beneficial or deleterious to their host cells in a natural state and how they impact physiology and fitness. For this work, we focus on the group of insertion sequence elements (IS), which are the most abundant group of transposable elements in bacteria. Using genome engineering techniques, we constructed strains with which we can look at the individual IS families (IS*1*, IS*3*, IS*5* etc.) and their mutants. The strains were validated using hybrid sequencing with Nanopore and Illumina and off-target mutations corrected. This allowed us to measure the biological impact of IS for bacterial growth and competitive fitness, by direct comparison of the strains in mono and co-culture experiments. We find that IS elements are directly beneficial to the physiology of a bacterium. They increase competitive fitness and growth rate. We thus identify a beneficial physiological role of transposon DNA in bacterial cells, providing an explanation for their abundance in bacterial genomes.

Keywords: bacteria, IS element, genome engineering

*Speaker

P elements in Drosophila willistoni group species

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The *P* transposable element invaded the *Drosophila melanogaster* natural populations between 1950 and 1970, following a horizontal transfer, likely from *Drosophila willistoni*. We analyzed by genomic DNA PCR and sequencing, the *P* element status in *willistoni* group species (*D. willistoni, D. equinoxialis, D. paulistorum, D. insularis, D. nebulosa, D. tropicalis*) in order to investigate what can be equilibrium states of the *P* family TEs on their long term in species (still active family or not). We found that potentially autonomous *P* elements appear to be still present in most of the *D. willistoni* and *D. equinoxialis* natural populations, whereas a large proportion of *D. paulistorum* and *D. tropicalis* collected lines apparently lack complete *P* copies. In addition, we have found in all except one more distant species (*D. nebulosa*) the presence of numerous *P*-MITEs (_˜200bp) with conserved sequences to be mobilized in *trans* and which appear to be identical by descent in all *willistoni* group species tested. They appear therefore to be old genome components present before species radiation. Strikingly, *D. insularis* appear to have retained only *P*-MITEs but no long *P* copies. We have found that most of the *willistoni* group species carry a common *P* element variant, named "protocanonical *P* element" (pc*P*), which differs from the canonical *P* element (c*P*) of *D. melanogaster* by three diagnostic marks including a SNP variation in the 3' Transposase Binding Site (TBS) which becomes therefore symmetrical with the 5' TBS, a situation distinctive from that of c*P* elements. Finally, small RNA sequencing in *D. willistoni*, *D. equinoxialis* and *D. tropicalis* ovaries showed the presence of sense and antisense *P*-homologous piRNAs, indicating a *P* repression based on a canonical germline piRNA mechanism. The evolutionary perspectives of the *P* family in the *willistoni* group species will be discussed.

Keywords: P elements, Drosophila willistoni, long term homeostasis

*Speaker

Evolutionary dynamics of Transposable Elements in Brachypodium distachyon based on a Pangenomic Approach

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The impact of Transposable Elements (TEs) in a genome can be explored by searching for their insertions. Individuals of the same species independently undergo TE insertions, causing inter-individual genetic variability. This variability between individuals is the basis of the natural selection that leads to an increased adaptation of individuals to their environment. A way to search for the potential role of TEs in host adaptation is through a pangenomic approach. The TE pangenome can be described by (i) TE insertions present in all individuals of the species (core-genome), (ii) insertions present only among a subset of individuals (dispensable-genome) or (iii) ecogenome when the individuals share the same environment, and finally (iv) individual-specific insertions.
A majority of current pangenome analysis methods are based on the alignment of reads from different genomes of the species to an assembled reference genome. But, the advent of the third-generation sequencing makes now possible to better approach this question using several *de novo* assembled genomes of the same species to avoid the bias introduced by a single reference genome. We have developed a new pipeline, called panREPET, to handle this type of data. This pipeline identifies copies shared by a group of individuals by comparing individuals pairwise.
We have described the pangenome in TEs of 54 *de novo* assembled genomes of *Brachypodium distachyon*. This pangenomic approach allows to improve the description of the evolutionary history of TE families and to date insertion events more precisely. We have also searched for factors affecting the evolutionary dynamics of TE families: we found that climate is a factor that may explain certain TE dynamics.

Keywords: pipeline, bioinformatics, pangenome

*Speaker

Is SINE and LINE transposable element evolution driven by nucleosome-positioning capacities?

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Nucleosomes are essential components of eukaryotic cells. A physical model of nucleosome formation showed that, for all vertebrate genomes analyzed, the positions of nucleosomes are in part encoded in the DNA sequence by Nucleosome Inhibitory Energy Barriers (NIEBs). This has been confirmed using experimental data from human (Drillon et al. 2016) and mouse (Tartour et al. 2023). In all cases, average GC-content profiles at NIEB borders display oscillations in phase with positioned nucleosomes, regions covered by nucleosomes being more GC-rich than NIEB and linker regions between nucleosomes.

We analyzed systematically all Dfam annotated TEs (Hubley 2015) in the human, mouse and pig genomes. We show that, for TEs having a polyA at their 3'end, this polyA is preferentially positioned on the interior side of a NIEB border. NIEB-associated TEs present GC-content oscillations that are similar to non-TE GC oscillations, excepted for the linker in human L1 retrotransposons and B2 mouse SINEs. We reconstructed the genome of the last common ancestors of human and chimpanzee, Mus musculus and Mus caroli, Sus scrofa and Sus cebifrons. We calculated the mutation rates since the divergence within all pairs of species, which was used to predict the GC content at equilibrium of TEs associated with NIEBs. For most NIEB-associated highly repeated TEs, we found that the current GC oscillation and the GC equilibrium are both correlated and in accord with nucleosome positioning around NIEBs. Interestingly, for both the B2 and L1 elements, GC at equilibrium at linker position showed a clear decrease that was not observed in current GC, indicating that these sequences are evolving towards the common GC oscillation pattern associated with NIEBs.

Taken together, our results suggest that TEs at NIEB borders have been shaped by mutations to present nucleosome-compatible GC-content oscillations, indicating a general link between chromosome structure and TE evolution.

*Speaker

Keywords: nucleosome, LINE, SINE, evolution, mouse, pig, human

The impact of transposable elements in ant evolution

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The ants have evolved a stunning global diversity with more than 15,000 extant species belonging to over 330 genera. Their ecological and evolutionary success is rooted in division of labour comparable to what somatic cells in a metazoan body achieve. The Global Ant Genomics Alliance (GAGA) has generated > 140 high-quality and contiguous genome assemblies, covering twelve of the world's 16 ant subfamilies. Combined with extensive natural history data, we have used these resources for exploring how transposable elements (TEs) have contributed to ant evolution. By comparatively studying abundance, diversity and distribution of TEs across the ant phylogeny, we identify significant contributions of transposable elements to the overall genome architecture and specific genomic and life history traits. Our analyses offer evidence for (1) transposon bursts coinciding with adaptive radiations in the three largest subfamilies of ants ¯60 mya, (2) an association of TE diversity with expansions of key gene families involved in the elaboration of social communication in ants, and (3) a division of ant genomes into two distinct compartments: fast evolving TE rich and slowly evolving TE poor regions that show divergent evolutionary trajectories and functional specializations. Together, these findings suggest a significant contribution of TEs as drivers of genomic change to the diversification and evolutionary success of ants.

Keywords: ants

*Speaker

Comparing the transposon landscapes of a putative ancient asexual and a sexual non-marine ostracod (Crustacea, Arthropoda)

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Ostracods are microscopic, bi-valved crustaceans with the best fossil record of all living arthropods. Their fossil record, starting 400 million years ago, together with a high prevalence of parthenogenetic reproduction and putative ancient asexuality, make non-marine ostracods fascinating evolutionary model organisms. In the absence of high quality ostracod reference genomes, we here compare transposon landscapes between two Illumina genome assemblies from the putative ancient asexual *Darwinula stevensoni* and the fully sexual ostracod *Notodromas monacha*. Both assemblies have around 60,000 contigs, sizes of 360-380 Mb, more than 100X coverage and BUSCO scores of 93 and 94%, respectively. Because homology-based programs are not sensitive enough to detect families of transposable elements (TEs) in species missing from Repbase or Dfam, we used three different pipelines for de novo analyses: REPET, RepeatMasker2 (RM2) and EarlGrey (RM2-based, with automated curation).
TE diversity between the two genomes differs substantially regardless which pipeline was used. The Illumina assembly of *N. monacha* is dominated by LTR retrotransposons (6.5%) with some DNA transposons (3.7%), whereas DNA (15.5%), LINE-like (5.9%) and rolling circle Helitron elements (1.5%) were most abundant in the assembly of *D. stevensoni*. Our results on the dominance of DNA (Tc/mar, hAT) and LINE-like (CR1, RTE) TEs in *D. stevensoni* parallel earlier findings from a partial genomic library, and differ from those of other asexuals. TE copies with a low number of nucleotide substitutions are only observed with REPET ("L" shape landscape)in both genomes.

Although the presented results may underestimate TE abundance, they indicate pronounced differences of the transposon landscapes and diversity between these two ostracod species. Analysis of related species should determine whether the differences are correlated with the reproductive mode or are lineage specific. We are currently curating TEs in an Oxford Nanopore draft assembly of *D. stevensoni* to further confirm our initial results.

Keywords: genome, asexuality, non, model organisms, de novo analysis, TE landscape and diversity

*Speaker

Functional Adaptations to their Host Underlie the Evolutionary Diversification of Endogenous Retroviruses

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Transposable elements profoundly affect the biology and evolution of their hosts, yet their own evolutionary dynamics remain poorly understood. Here, we investigate insect endogenous retroviruses (iERVs), a monophyletic group of LTR retrotransposons that have acquired the trait of infectivity, likely through capture of a Baculovirus *envelope* gene. In *Drosophila* ovaries, iERVs with functional *envelope* have adapted their expression to any somatic cell type, from where they infect the germline. Strikingly, related retroviruses show distinct expression patterns, indicating niche partitioning. In contrast, all non-infectious iERVs that emerged through secondary *envelope*-loss are specifically expressed in the germline. Using transgenic reporters *in vivo* and sequence analysis of multiple iERV lineages including the variants of the transition element *rover,* we elucidate how this discrete co-variation evolved via changes in *i)* the elements transcriptional *cis*-regulatory sequences and *ii)* their functional envelope status (intact/defective). Notably, **the genome-protecting piRNA pathway - co-evolving with iERVs - has assimilated iERV promoter and sequence information into piRNA clusters, underscoring the functional significance of iERV expression in somatic niches. We propose that the evolutionary innovation of cell-to-cell infectivity gave rise to the iERV ancestor which then diversified through trait diversification and antagonistic virus-host interactions, processes that likely underpin niche-specific expression of endogenous retroviruses in vertebrates as well.**

Keywords: gypsy, Drosophila, evolution, envelope, infectivity

*Speaker

The pangenomic approach enables a better understanding of the dynamics of transposable elements in the human genome.

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3,4

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Humans originated on the African continent and migrated out of Africa, requiring adaptive processes. Patterns of transposable element (TE) variation in populations can be used to trace the evolutionary history of our species. Comparative analyses can therefore be expected to reveal signatures of selective forces in TEs that indicate potential adaptive loci. However, the evolutionary dynamics of TEs during the out-of-Africa event has not been well studied. We took advantage of the promising recent human pangenome reference, built using long-read sequencing and variation graphing of 47 human individuals from eight populations, which improved the detection rate of large variations without reference bias. The aims of our study are (1) to characterise the frequency distribution of TEs along the pangenome in the world population and possible factors influencing their evolution; (2) and to examine ET loci under positive selection using genome-wide frequency and haplotype-based selection analyses as a pairwise comparison between African and non-African populations.Our initial results show a non-random distribution of TE across chromosomes, with an over-representation in intergenic and intronic regions, mostly detected as very rare TE insertions. Recombination rate and distance between TEs have little or no effect on their frequency spectrum. As TEs also show patterns of population stratification, we were able to identify TE loci containing putative candidate genes under positive selection. Candidate genes were associated with immune, metabolic and developmental pathways and functions. All of these elements suggest features of local human adaptation. Further analysis will be required to achieve a more robust functional understanding of human evolution.Taken as a whole, our study provides evidence for the relevance of pangenomics in elucidating TE dynamics in humans. Future larger sampling with more samples per population will allow a better resolution of genetic diversity and study of the impact of TEs to such evolutionary processes.

*Speaker

Keywords: human, pangenomics, genomics, evolution

Identification of Transposable Element families from pangenome polymorphisms with pantera

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Most methods used to identify TEs in a newly sequenced genome are based either on matches to known families from other species, or on them being repeated at high copy number. As species with more than one high quality assembly become more common, including those from diploid individuals with both haplotypes assembled, an alternative strategy becomes possible, in which we focus on the polymorphic character of TEs caused by their mobility. We present a method, Pantera, that uses structural polymorphisms found in pangenomes to create a library of TE families recently active in a species, or in a closely related group of species. This approach is particularly strong for finding full length TE sequences, and low copy number families. We will show the results of applying pantera both to well-studied species with curated TE libraries and also to a wide range of species from the Darwin Tree of Life project. As an illustration, we will discuss more than 700 Mavericks identified in 411 Lepidoptera species, which fall into at least four groups that are differentiated by sequence homology, internal structure and length distribution.

Keywords: pangenome, denovo library, maverick, Darwin Tree of Life, methodology, pantera, tool, Lepidoptera

*Speaker

Contribution of transposable elements to the host shift in cactophilic Drosophila species

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Insects that use plant hosts as feeding and breeding sites rely on this interaction for their survival. In the process of exploring different hosts and particularly when host shift occurs, successive adaptations might initiate reproductive isolation between populations, as a result of divergent selection. Therefore, host shift has been considered an important hallmark for ecological speciation. The adaptations underlying shifts have been attributed to both genomics and transcriptomics variation in many species. Despite the well-known contribution of transposable elements (TEs) as foremost engine for genome evolution, extensively contributing to local adaptation between species/populations, the extent of TEs' contribution to host shift remained unexplored. Cactophilic *Drosophila* species are an excellent model to investigate the genetic background underlying host shift since many species have different primary hosts. In this work, we selected seven cactophilic *Drosophila* species with different host preferences and incipient reproductive isolation to investigate the role of TEs on their divergence. Using long reads sequencing technology, we observed differential expansion of TE families among species, as well as a high prevalence of TE copies in the promoter region of genes associated with host location. Several insertions are likely to harbor transcription factor binding sites, highlighting their potential adaptive role. In addition, our transcriptomics analyses from head and larvae tissues have shown the presence of polymorphic TE insertions generating transcripts derived from genes and TEs (chimeric transcripts), including genes associated with host shift. Taken together, our results demonstrate that TEs have substantial contribution to both genomic and transcriptomic variability of cactophilic *Drosophila* species, even between recently (_˜200,000 years) diverged subspecies, reinforcing their role in rapid evolution. Our combined genomics and transcriptomics approaches provide new insights regarding the role of TEs in the evolution of host shift, and ultimately their contribution to ecological speciation.

Keywords: host shift, ecological speciation, chimeric transcripts

*Speaker

TEs as a major driver of genomic variation in trees ? Jumping MITEs in common beech revealed by TIPs and eccDNA

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Transposable elements (TEs) are predominant in most plant genomes but their ongoing activity and diversity in plant populations has been mostly characterized in annual species. Beech trees (*Fagus sylvatica*) make up most of European forests and are highly threatened by drought and climate change. We sought to investigate the genomic diversity caused by TEs and their adaptive potential in this long-living species. We characterized TE insertion polymorphism (TIPs) in a single population of 150 common beech trees from the preserved Massane forest, a UNESCO world heritage site located near Perpignan, France. Using 672 and 558 LTR (Long Terminal Repeats) retrotransposon and MITE (Miniature Inverted- repeat Transposable Element) families, respectively, we show that the majority of TIPs are highly polymorphic and mostly privately shared within the population. We conducted a Genome-Wide Association Study (GWAS) using these TIPs as markers and identified several candidate TIPs associated with bud burst date, implying their potential involvement in regulating this important phenotypic trait. TIPs distribution revealed several hotspots near stress responsive genes. Bud burst date being an adaptive trait, our results could open new perspectives for the selection of best adapted trees.

Furthermore, given this high level of recent TE activity in common beech, we investigated ongoing TE mobility using extrachromosomal circular DNA (eccDNA) sequencing or mobilome-seq. We detected several active TEs in different beech trees from the Massane and Verzy French forests. Notably, we identified an highly active MITE family, responsible for somatic mutations. We further found several other active MITEs producing abundant eccDNA suggesting that MITEs could play a role in genome dynamics in this species. All together, our study sheds lights on how TEs impact genome evolution and adaptation in a non model perennial species. This could help us gain insights on TE dynamics and amplification *in natura*.

*Speaker

Keywords: Extrachromosomal circular DNA (eccDNA), Transposable Element insertion polymorphism (TIPs), Plants/Trees genomics and adaptations, Climate change, Active TEs, MITEs, LTRs

B chromosome repetitive composition and interference in germline expression of Transposable Elements and genes: insights from Drosophila melanogaster

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The standard chromosome set of individuals includes autosomes and, frequently, sex chromosomes. However, certain species harbor non-essential chromosomes known as B chromosomes, which can influence cell metabolism by affecting gene expression. Studies in *Drosophila melanogaster* identified B chromosomes that are predominantly heterochromatic and likely originated from Chromosome 4. Here we advanced in detail about the repetitive composition of B chromosomes and impact of B chromosomes on the expression of genes and Transposable Elements (TEs) located on standard chromosomes. Using tools like RepeatExplorer and RepeatMasker, we quantified the abundance and divergence of repetitive sequences from genomes B+/0B and from the B chromosome isolated by flow cytometry. Fluorescent *in situ* hybridization (FISH) was subsequently used to understand sequence-sharing patterns between B and standard chromosomes. RSEM, TEspeX, Deseq2, and EdgeR were used to quantify and perform differential expression analysis of coding transcripts and TEs. Overall, the isolated B chromosome is composed of approximately 17.04% TEs, 16.88% satellite DNAs (satDNAs) and microsatellites, and 1.9% multigene families, totaling 35.82% of repetitive composition. FISH results showed no sequence was shared between the Bs and other chromosomes besides 4th, supporting the B chromosome's origin from the 4th chromosome. Concerning the expression analysis, the addition of a B chromosome to the 0B genome exhibited a minimal immediate effect on coding transcripts (0.8%), which after approximately 30 generations resulted in a substantial increased effect (7%). Interestingly, TEs displayed differential expression following B chromosome addition (7.3%), maintaining a consistent effect (8.5%) over generations. Moreover, TEs were more susceptible to B dose (5.8% by B dose and 12.4% by presence) compared to coding transcripts (0.7% altered by B dose and 9.8% altered by presence). These findings shed light on the dynamic interactions between B chromosomes and the genomic landscape, offering insights into their role in gene and TE expression modulation.

*Speaker

Keywords: cytogenetics, extra chromosome, TEspeX, differential expression

Teaching transposon classification as a means to crowd source the curation of repeat annotation – a tardigrade perspective

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The advancement of the third-generation sequencing era is providing an unprecedented opportunity for the global genomic community. Because of the increasing cost-effectiveness of long-read sequencing, an exponential number of high-quality genomes are being released rapidly for non-model organisms, providing valuable resources for conservation and evolutionary studies. Within this context, the significance of in-depth analyses of repetitive elements, particularly transposable elements (TEs), is increasingly recognized in understanding genome evolution. However, while a plethora of bioinformatic tools is available for identifying and annotating TEs, any automated annotation effort is constrained by the phylogenetic distance of the target species from a curated and classified database of repetitive element sequences. Furthermore, manual curation of raw repeat libraries is deemed essential due to the frequent truncation and incomplete status of automatically generated consensus sequences. Nonetheless, manual curation and classification are time-consuming processes, they offer limited short-term academic rewards, and are typically confined to a few research groups where they are taught through hands-on experience. Crowdsourcing efforts could offer a significant opportunity to bridge this gap and empower the scientific community with high-quality, reusable genomic resources. Here, we present an example of such a process with both in-person and online courses, each attended by one or two dozen participants. These courses focused on tardigrades, a phylum for which no TE libraries existed, and nearly all repeats were previously annotated as "Unknown". After a series of theoretical lectures on TEs biology and practical examples of manual curation, participants were divided into groups curating hundreds of consensus sequences each. After a peer-review process we discovered a huge diversity especially of non-autonomous TEs, demonstrating that a peer-reviewed classroom setting can yield substantial benefits for both students and for the entire scientific community. A hidden treasure awaits discovery within non-model organisms.

Keywords: manual curation, genome evolution, crowd sourcing

*Speaker

LTRpred2: De novo annotation of intact LTR-retrotransposons across the Eukaryotic Tree of Life

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Eukaryotic genomes are typically full of long terminal repeat-retrotransposons (LTR-RTs). The mobility of LTR-RTs alters genome structure and can thereby generate phenotypic novelty by altering gene function. LTR-RTs have a well-defined structure which makes them particularly suitable for computational detection. This fact has permitted the development of a diverse range of scientific softwares and pipelines for their de novo annotation through structural identification. We have tested some of the existing softwares and found a high discordance between their outputs. Moreover, visual inspection of the datasets showed that these tools identify a certain amount of false positive sequences. To overcome these limitations we introduce LTRpred2, which combines several methods for de novo structural detection of LTR retrotransposons to enhance annotation sensitivity and select only the most confident candidates using protein domain search. LTRpred2 also implements functions to perform a vast array of analyses including metagenomic studies comparing retrotransposon family activity across kingdoms, copy number quantification, retrotransposon family clustering, etc. We use LTRpred2 to re-annotate and explore the LTR-RT landscape across genomes of the entire Eukaryotic Tree of Life in order to uncover the structural biodiversity of these TEs and find novel correlations with genomic and environmental traits.

Keywords: annotation, LTR, retrotransposons

*Speaker

Investigating the impact of transposons on complex brain functions and behaviours

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The Treiber Lab launched in January 2024 and we are set to investigate how intronic transposon insertions that frequently introduce cryptic splice sites within neural genes change proteins, brain functions and behaviours. I will present high-throughput single-cell transposon expression data of the Drosophila melanogaster brain, which reveals that most transposon families exhibit highly complex, non-random expression patterns. We show that co-expression with neighbouring genes accounts for the majority of the observed transposon expression. In addition, we find transposons that are specifically up-regulated when animals form new memories, and this activity-dependent transposon expression matches that of neighbouring genes. My presentation will highlight how new technological advances in long-read single-cell sequencing will help unravel how transposon expression in the brain impacts an animal's behaviour.

Keywords: transposon expression, single, cell sequencing, transposon gene chimera, alternative splicing

*Speaker

Back to TE future: Understanding the fitness effect of transposable element mobilization under climate change scenarios

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Although most mutations induced by transposable element (TE) insertions are likely deleterious, empirical and theoretical evidence indicate that a continuum of selective effects must exist. However, because transposition is typically rare, assessing their rate, landscape, and consequences is extremely challenging. We have developed a TE display followed by high-throughput sequencing method (TED-seq) that enable us to detect new insertions with ultra high-sensitivity. Using this technique, we are investigating the fitness effects of TE insertions in large populations of *Arabidopsis thaliana* plants experiencing an early TE invasion. In particular, we are performing competition experiments in realistic environments reproducing nowadays or climate projections for the end of the 21st century under an extreme greenhouse gas emission scenario. Following each generation, seed samples are collected and bulk for TED-seq analysis, allowing us to measure transposition rates as well as to track population frequencies of each TE insertion. Our study of the first two generations revealed significant variation in transposition rates between present and future climates, as well as numerous TE insertions presenting signatures of positive selection. Our results shed new light on the environmental pressures shaping transposition activity and its potential role in adaptation to future environments.

Keywords: transposition fitness, population genomics, climate change

*Speaker

MITE-driven dissemination of regulatory elements in the maize genome

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Due to their repetitive nature and the fact that they harbor regulatory elements, transposable elements (TEs) were early envisioned as drivers of the evolution of gene regulatory networks through their ability to spread 'pre-built' regulatory elements in the genome. These first models have been recently refreshed by recent large-scale analyses of eukaryotic genetic regulatory landscapes, showing that TEs from various superfamilies can participate in the rewiring of different gene regulatory networks involved in key biological functions in various eukaryotic species. Nevertheless, the molecular mechanisms underlying the generation of TE-driven networks have been only characterized for a few cases and remains to be investigated at a larger scale, in particular in plants which have lagged behind animal studies. In a recent study on maize (Fagny et al., 2021; doi.org/10.3389/fgene.2020.606285), we used a systems biology approach to investigate the enhancer-driven regulatory network of two tissues at different stages: leaves at seedling stage and ear-covering leaves at male flowering (husk). We have shown that husk-specific enhancers are enriched in MITEs, among which *Pif/Harbinger* elements harbor new Transcription-Factor Binding Sites. We will present how *Pif/Harbinger* sequences have contributed to the dissemination of potential regulatory elements throughout the maize genome and which functions they may have participated to.

Keywords: Gene regulatory networks, cis, regulatory elements, MITE, maize

*Speaker

Microbial single-cell RNA sequencing to investigate environmental triggers for ICEclc transfer competence activation in Pseudomonas

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Horizontal gene transfer (HGT) is the process by which DNA is transferred from a donor cell to a recipient that is not part of its progeny. Mechanisms of HGT in prokaryotes include transformation, transduction, and conjugation; the latter of which transfers plasmids or chromosomally excised DNA like integrative and conjugative elements (ICEs) from a donor to a recipient in a cell-to-cell dependent contact. ICEs are widespread autonomous mobile DNA, containing the genes necessary for integration, excision, and the conjugative machinery. ICE*clc* of *Pseudomonas putida* is our experimental model and is characterized by the presence of the *clc* genes for chlorocatechol degradation. Its transfer is initiated from a subpopulation of cells (3-5%) that become transfer competent (tc). The proportion of tc cells is the highest when cultures have been grown on 3-chlorobenzoate (3CBA) as a sole carbon source and when they enter stationary phase. However, the link between growth on 3CBA and ICE*clc* transfer competence activation is unknown. Our aim is to better understand the influence of environmental factors on ICE*clc* activation, assuming that growth on 3CBA induces metabolic pathways in *Pseudomonas* which relate to tc cell formation. To identify such potential pathways, we use microbial single-cell RNA sequencing (scRNAseq) and quantify differences in transcript abundances in single cells growing on different carbon sources. We were able to differentiate tc- from non-tc cells based on their transcriptional fingerprints, and further detected carbon source and growth phase signatures. Tc cell-specific expression signatures identified by scRNAseq will be followed up in genetic and reporter studies.

Keywords: Integrative conjugative elements, ICEclc, Pseudomonas, single cell RNA sequencing

*Speaker

TEs in plant-microbe interaction revealed from total RNA-seq approach

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Strategies to underpin plant-microbe interaction have benefited from RNA-seq approaches to reveal plant genes that respond to the presence of a microbe, be it a beneficial organism or a pathogen. Most studies rely on model plants and well-known interactions. In addition, most transcriptome data available is derived from mRNA-captured molecules. Towards enlarging the opportunities from a given tissue sample, a total RNA-seq approach is devised in a non-model polyploid organism such as sugarcane. A total transcriptome prepared from 10 interacting conditions reveals significant changes in TE expression and opens avenues for identifying TE indicators. The experiment explores four sugarcane cultivars submitted to different pathogenic conditions, and tissues are collected at 48 post-inoculation. Differentially expressed TEs are identified from a previously built reference sugarcane TE database. Both transposons and retrotransposons respond to the inoculation but eight elements. Helitron-derived elements are the most affected TEs. Maximus-derived elements are mostly not responsive. Downregulation of TEs is observed in two of the three susceptible cultivars, while the tolerant cultivar modulates the expression of the whole TE family assortment. Some of the identified lncRNAs are derived from previously described Transposable Elements. The work presented sheds light on a multiscale TE interactome not previously projected by classical mRNA-targeted transcriptome sequencing.

Keywords: plant, microbe interaction, sugarcane, total transcriptome

*Speaker

CAULIFINDER: a package for the automatic detection and annotation of endogenous viral sequences of Caulimoviridae

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Endogenous viral sequences (EVEs) result from the integration of full or part of viral genomes into the genome of eukaryotic or prokaryotic hosts. EVEs sometimes represent a significant part of the host genome. In addition, they provide access to unique viral sequences that can be useful in paleovirology approaches to study the evolution of viruses over time steps through several million years. However, EVEs are frequently ignored in genome annotation due to the lack of dedicated bioinformatics tools for their detection and characterization, which have long relied on manual processes. In plants, most of the known EVEs belong to the family Caulimoviridae, the only family of retrotranscribed plant viruses. However, Caulimoviridae EVEs (ECVs) are often confused with transposable elements owing to their similarity with Ty3/Gypsy elements (family Metaviridae). Here we present "CAULIFINDER", a bioinformatics software package for the automatic characterization and annotation of ECVs in plant genomes. CAULIFINDER consists of two complementary pipelines. The first one detects repetitive ECVs and performs a reconstruction of consensus sequences. The consensus sequences are then used to annotate all matching ECV copies in the analyzed genome. The second pipeline searches for the ECV marker gene 'reverse transcriptase' in a given plant genome and integrates the representative sequences in a phylogenetic tree of the Caulimoviridae, allowing the diversity of ECVs to be assessed regardless of their copy number. CAULIFINDER is a versatile tool for producing an annotation of ECVs in plant genomes and for collecting fossil sequences that can be used to conduct evolutionary studies of Caulimoviridae using paleovirology approaches. It is distributed in a Docker open-access image. We will present the design and evaluation of CAULIFINDER, carried out on sequence data from several plant genomes, and discuss the generic scope of our work for studying the evolution of viruses over long time steps.

*Speaker

Keywords: Ortervirales, Caulimoviridae, Caulifinder, pipeline, Endogenous viral elements, genome annotation, paleovirology

Small ruminants versus endogenous retroviruses: an evolutive showdown still in progress in the domestic goat genome

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Endogenous retroviruses (ERV) are LTR retrotransposons derived from ancient retroviral infections. In small ruminants, unlike in most species, an ERV family coexists with its exogenous counterparts. Apart from this family, no comprehensive study has been carried out on sheep and goat ERVs, and their co-evolutionary history with their host genomes remains largely unknown.
In this study, we characterized 23 class I and II ERV families across four reference assemblies of domestic and wild sheep and goats, as well as one cattle assembly. Among these families, 15 were found to be shared among all ruminants, 6 were exclusive to small ruminants and 2 to cattle. The presence of these families has been assessed in other ruminant species, revealing different integration events before the *Bovidae* speciation 17 million years ago and more recently from 6 to 14 million years ago.

Between 0.5 to 1 % of small ruminant genomes were annotated as ERVs. Although class I ERVs showed comparable profiles between species, contrasting evolutionary dynamics were identified for class II ERVs. A prevalent family in the cattle genome, family-1, were only present as relics in small ruminants. Two other class II families designated as families-3 and -5, showed more abundant and conserved copies exclusively in domestic goat. Notably, family-5 is the family closely related to circulating exogenous retroviruses and was identified with 22 copies featuring fully identical LTRs and 7 having complete coding capacities. These findings collectively suggest that transposition and endogenization events may still be occurring in the domestic goat genome.
This study is the first comparative analysis of small ruminant ERVs and revealed that ERV families were shared among species but with lineage-specific insertion dynamics. Thus, small ruminants offer an incomparable model to compare the factors implicated in ERVs' expansion

*Speaker

and decline across genomes.

Keywords: Endogenous retroviruses, small ruminants, evolution, insertion dynamics

Transposable elements in X-linked genes contribute to misexpression in Drosophila mojavensis and D. arizonae hybrids

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Hybrid sterility is a fast-evolving postzygotic mechanism that can reduce gene flow between incipient species. Regulatory networks divergence has been proposed as a potential mechanism underlying it. TE insertions have been identified as sources of regulatory sequences driving expression divergence in several biological contexts, such as the male germline. This study aims to identify if TE insertions in the regulatory regions can contribute to gene deregulation of sterile hybrids. For this, we took advantage of the recently diverged sister species *Drosophila mojavensis baja* and *D. arizonae*, that produce male sterile and fertile hybrids. Although the male sterile phenotype is attributed to multiple deregulated genes in germline, genes linked to the X chromosome have been demonstrated as major factors. We found that X chromosomes are enriched in TEs and in genes containing TEs for both species. X-linked genes were upregulated in the testis of sterile hybrids, in contrast to the fertile ones. These genes were enriched in TE insertions in their regulatory regions. Moreover, for X-linked upregulated genes in both hybrids and downregulated specifically in sterile hybrids, we also found an enrichment for TEs. However we found overall depletion of insertions for the autosomal genes. Our results suggest that TE insertions might play an evolutionary role in the divergence of the regulation of X-linked genes, that can lead to hybrid genome incompatibilities and, therefore, to sterility for these species.

Keywords: Hybrid sterility, Genomic incompatibilities, Regulatory divergence, Gene deregulation, Male germline

*Speaker

Oxford Nanopore Sequencing reveals complex mechanisms of repetitive DNA propagation in Tribolum castaneum

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The beetle *Tribolium castaneum* is a worldwide pest of stored products and representative of the most species-rich animal order on earth, the Coleoptera, and is the first sequenced beetle species. As its evolution is more representative of insects than that of *Drosophila melanogaster*, *T. castaneum* has become one of the most important models in the field of insect evolution, physiology and development. Even though experiments and sequencing analyses have shown that the *T. castaneum* genome is rich in different types of repetitive DNA sequences, most remain underrepresented in previous genome assembly versions, limiting analysis of their distribution, movement and rearrangement in the genome. The main obstacle to uncovering these properties of repetitive DNA is the relatively short read length used in conventional genome assembly pipelines. Therefore, we combined the results of Oxford Nanopore long-read sequencing with the existing reference genome to generate a new, high-quality genome assembly (TcasONT) of the model beetle *T. castaneum*. The resulting genome assembly is enriched by 50 Mb in the repetitive genome part, which corresponds to 25% of the estimated genome size of *T. castaneum*. Therefore, we utilized the expanded assembly to perform global and in-depth analyses of different classes of transposable elements (TEs), the abundant non-(peri)centromeric satellite DNA (satDNA) and their relationships. Although we found that TEs and satDNA often do not overlap, we showed there are specific cases where certain satDNA variants fuse with TEs to create a new repeat unit that can propagate independently in the genome or possibly by extrachromosomal circular DNA. Additionally, we have confirmed that the presence of both TEs and satDNA is not restricted to specific non-coding parts of the genome but rather propagates throughout euchromatin, suggesting an efficient mechanism by which repetitive DNA can have direct impact on gene regulation.

Keywords: oxford nanopore sequencing genome assembly

*Speaker

Analysis of TE population in polyploid oat reveals subgenome specific activity patterns

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Oat (*Avena sativa*) is an allohexaploid cereal comprising three large and highly repetitive subgenomes (A, C and D). The C subgenome separated _˜8 million years ago (MYA) while A and D separated _˜3.5 MYA. We analyzed over 22.000 full length retrotransposons belonging to four high-copy and two centromere-specific families. Out of these, we found four families to be subgenome specific. The analyzed TE families were active during different times in *A. sativa* evolution, allowing to study how they evolved in different species lineages. For example, we found two *Gypsy* families with a distinct activity pattern differing between the C subgenome and the A and D subgenomes. Additionally, detailed characterization of subgenome-specific TEs showed them to be excellent markers for chromosomal translocations that occurred after polyploidization events.
The *Copia* family *RLC_Angelina* is highly abundant in all three subgenomes. Interestingly, we identified two *RLC_Angelina* sub-populations one comprising autonomous and one non-autonomous elements. This demonstrates that pairs of autonomous and non-autonomous TEs can persist over millions of years. In the A and D genome the *RLC_Angelina* family went mostly silent before their separation and in the C genome before the tetraploidisation that brought together the C and D genomes _˜1.5. MYA. (CCDD).
In addition, we identified the *Gypsy* family *RLG_Cereba* in the centromeres of the A and D subgenomes while we found a different family, *RLG_Ava*, to dominate the C subgenome centromeres. Interestingly, the *RLG_Cereba* family was absent in the C subgenome and *vice versa* for the *RLG_Ava* family. This suggests a competition between centromere specific retrotransposon families.
Surprisingly, we found no evidence of TEs specifically activated after polyploidisation events. On the contrary, we identified TE families that were highly active before the polyploidisation events, but went silent after. This challenges the notion that polyploidization is followed by a "genomic shock".

Keywords: Avena sativa, Retrotransposons, Genome Evolution

*Speaker

Regulatory evolution of inflammatory response through the lens of primate-specific transposable elements

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Background:
Inflammation is crucial for survival and linked to many diseases. Its evolutionary adaptation to environmental challenges is largely driven by genomic enhancers. Tracking the evolution of these enhancers is key for understanding the modern regulatory architecture of inflammatory response.

Results

We traced the evolution of DNA sequences in human enhancers back to macaques, identifying distinct categories: slow-evolving enhancers that are orthologous with macaques, and fast-evolving enhancers marked by variations exclusive to either human-chimpanzee lineage or to humans alone, respectively. Fast-evolving enhancers, unlike their slow counterparts, are enriched in 120 subfamilies of primate-restricted transposable elements (pTEs) and predominantly associated with inflammation-related transcription factor (TF) motifs, some of which have uniquely expanded in great apes. pTEs contribute to the formation of these motifs, occasionally being nearly the sole source of their novel expansion, notably for NFkb. Fast-evolving enhancers show a stronger link to GWAS risk variants for inflammatory and autoimmune diseases compared to slow-evolving ones. Population genetics analysis revealed that the mere presence of pTE in enhancers, especially those linked to inflammatory disorders, increases the likelihood of positive selection. Enhancers under positive selection in modern populations tend to be shared across different tissues and, while linked to immune and developmental functions, are enriched in inflammation-related TF motifs.

Conclusion

The unique contribution of pTEs to rapidly evolving enhancers highlights a novel mechanism potentially influencing the inflammatory response since the divergence of the great apes. Inflammation-associated cues in enhancers may have evolved more rapidly than other types of regulation. By associating with highly adaptive enhancers, pTEs could have offered a distinct advantage under selective pressures related to inflammation.

Keywords: Transposable elements, inflammation, evolution

*Speaker

Mobile element insertions and structural variation in a large human population reference database

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Structural variants (SVs) are a numerous and inadequately characterized source of human genetic variation that occur from a diverse set of molecular mechanisms. They include large deletions, duplications, inversions, mobile element insertions (MEIs), and more. Using our state-of-the-art SV calling pipeline, GATK-SV, we have identified over 1.1 million high quality SVs from 63,046 unrelated individuals with whole genome sequencing (WGS) data, newly released as part of the Genome Aggregation Database (gnomAD). Within the gnomAD v4 callset, we identify over 200,000 MEIs, comprising 173,374 Alu, 30,223 L1, and 17,607 SINE-VNTR-Alu (SVA) element insertions, the largest high-quality callset of MEIs released to date. We examined distributional patterns of MEIs across the genomes and gene content, as well as their frequency within different functional annotation categories. We also identified variant distributions within many different sets of noncoding annotations from numerous sources. Additionally, we use the GATK-SV variant calls within the newest release of the GTEx dataset, which consists of paired WGS and transcriptome data from 851 individuals across 54 tissues. Upon identifying expression and splicing quantitative trait loci, we put forth the largest set of common MEIs that are likely causal for changes in expression and splicing.

Keywords: population genetics, structural variant, Alu, human genetics, gnomAD, database

*Speaker

Transposon-mediated genic rearrangements underlie variation in small RNA pathways

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Transposable elements (TEs) are parasitic DNA sequences that insert into the host genome and can cause alterations in host gene structure and expression. Host organisms cope with the often detrimental consequences caused by recent transposition and develop mechanisms that repress TE activities. In the nematode *Caenorhabditis elegans*, a small interfering RNA (siRNA) pathway dependent on the helicase ERI-6/7 primarily silences long terminal repeat retrotransposons and recent genes of likely viral origin. By studying gene expression variation among wild *C. elegans* strains, we discovered that structural variants and transposon remnants at the *eri-6/7* locus alter its expression in *cis* and underlie a *trans*-acting expression quantitative trait locus affecting non-conserved genes and pseudogenes. Multiple insertions of the *Polinton* DNA transposon (also known as *Mavericks*) reshuffled the *eri-6/7* locus in different configurations, separating the *eri-6* and *eri-7* exons and causing the inversion of *eri-6* as seen in the reference N2 genome. In the inverted configuration, gene function was previously shown to be repaired by unusual *trans*-splicing mediated by direct repeats flanking the inversion. We show that these direct repeats originated from terminal inverted repeats specific to *C. elegans Polintons*. This *trans*-splicing event occurs infrequently compared to *cis*-splicing to novel downstream exons, thus affecting the production of ERI-6/7. Diverse *Polinton*-induced structural variations display regulatory effects within the locus and on targets of ERI-6/7-dependent siRNA pathways. Our findings highlight the role of host-transposon interactions in driving rapid host genome diversification among natural populations and shed light on evolutionary novelty in genes and splicing mechanisms.

Keywords: transposon, structural variation, inversion, splicing, small RNA, C. elegans

*Speaker

Exploring the Role of a CENPBL Gene Family in Paramecium Genome Rearrangements

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Paramecium tetraurelia harbors two distinct nuclei in its cytoplasm. The micronucleus (MIC, 2n) contains the germline genome that is transmitted to the offspring at each sexual cycle. The somatic macronucleus (MAC, _˜1600n), responsible for gene transcription, is destroyed during sexual processes while a new MAC differentiates from a copy of the MIC. During MAC development, _˜30% of the germline DNA is eliminated: transposable elements (TEs) and minisatellites are eliminated imprecisely, whereas 45,000 TE-derived Internal Eliminated Sequences (IESs), dispersed throughout the germline genome, are excised precisely from coding and non-coding regions to reconstitute functional genes. IES excision depends upon PiggyMac (Pgm), which cleaves DNA at IES ends, and five Pgm-like partners. We recently characterized the developmental timing of programmed DNA elimination (PDE) genome-wide and identified a distinct and reproducible elimination timing for IESs. We proposed that sequential PDE may provide *Paramecium* with a unique mechanism to fine-tune zygotic gene expression as PDE progresses. This hypothesis is illustrated by our discovery of a family of *CENPB-like* genes (*CENPBL*) which can only be expressed from the new MAC at an early stage of its development, because parts of their coding and/or regulatory sequences are excised at later stages by the IES excision machinery. *CENPBL* genes are therefore expressed only during the time window when IESs are present. CenpbL proteins share similarities with human CENP-B and its homologs, which derive from domesticated Pogo transposases and are involved in centromeric heterochromatin formation and TE silencing. Intriguingly, *Paramecium* CenpbLs have lost the DDE superfamily endonuclease domain, while retaining the CENPB-type DNA-binding HTH domain. We will present the results of RNA interference experiments designed to phenotypically characterize the effect of *CENPBL* knockdowns during MAC development and investigate their potential link with PDE.

Keywords: ciliates / programmed DNA elimination /domesticated transposases / CENPB, related proteins

*Speaker

Real-time observation of uptake of mobilisable elements during bacterial experimental evolution

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Bacteria living in communities can acquire new genes by horizontal gene transfer (HGT) mediated by mobile genetic elements. HGT events are typically identified by comparative genomics, and experimental observation of events remains rare. We have filled this gap with a direct experimental approach to observe HGT. We propagated the bacterium *P. fluorescens* SBW25 by serial transfer, and added a sample of filtrate devoid of bacteria from a garden compost community to these cultures. By sequencing random colonies after several transfers, we successfully detected the uptake of three foreign elements into the SBW25 genome. The elements are _˜23kb, _˜44kb and _˜55kb in length, and were inserted at the same genomic position, downstream of *tmRNA*. The MGE classifiers we used returned no consensus in how to categorise the function of genes carried by these elements. The integrated sequences all start with a tyrosine integrase, followed by various putative phage defence systems and end with a homologous stretch of DNA encoding lambda repressors and helicases. How does this new type of MGE transfer and what – if any – benefit it might provide to its new host? I55 (the 55 kb element) was able to circularise spontaneously, but it transferred between SBW25 cells only in the presence of community filtrate. Deletion of the tyrosine integrase fixed I55 in the genome and disabled transfer between cells. Also, I55 has a significant fitness advantage over the wild type in presence of the community filtrate. We suggest the intercellular transfer and fitness benefit of I55 crucially depends on bacteriophages present in the community filtrate. A study of the exact genes in I55 that confer the fitness advantage is currently underway. Our approach has allowed us to witness HGT events in real-time, resulting in identification of a new type of MGE that potentially protects its host from phage attack.

Keywords: Bacteria, Horizontal Gene Transfer, Bacterial Genetics, Bacterial Evolution, Experimental Evolution

*Speaker

TEs in health and disease

Hepatitis B virus polymerase restricts LINE-1 mobility

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Long interspersed element-1 (LINE-1, L1) transposable element (TE) composes about 17% of the human genome. However, genetic and biochemical interactions between L1 and hepatitis B virus (HBV) remain poorly understood. In this study, I found that HBV restricts L1 retrotransposition in a reverse transcriptase (RT)-independent manner. Notably, HBV polymerase (Pol) strongly inhibited L1 retrotransposition. Indeed, the ribonuclease H (RNase H) domain was essential for inhibition of L1 retrotransposition. The L1 ORF1p RNA-binding protein predominantly localized into cytoplasmic RNA granule termed P-body. However, HBV Pol hijacked L1 ORF1p from P-body through an interaction with L1 ORF1p, when both proteins were co-expressed. Furthermore, HBV Pol repressed the L1 5' untranslated region (UTR). Altogether, HBV seems to restrict L1 mobility at multiple steps. Thus, these results suggest a novel function or activity of HBV Pol in regulation of L1 retrotransposition.

Keywords: HBV, LINE, 1, retrotransposition, polymerase, reverse transcription, RNase H

*Speaker

Role of LINE-1 promoter methylation in the development of PTSD in a mice model

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Post-traumatic stress disorder (PTSD) is a neuropsychiatric disorder characterized by changes in behavior and personality. Long Interspersed Nuclear Element 1 (LINE-1) is a retrotransposable element expressed in early development that is later repressed. Adult expression of LINE-1 is active under pathological conditions, and evidence in humans show a correlation between PTSD patients and LINE-1 expression. Here we aimed to understand if LINE-1 expression influences the development of PTSD in mice. Single Prolonged Stress (SPS) was used to induce PTSD in P60 mice. PTSD development was determined by behavioral testing. Changes in LINE-1 expression were determined by RT-qPCR in hippocampus and cortex. Methylation of the DNA promoter sequence of LINE-1were performed by bisulfite sequencing. Induction of PTSD by SPS shows that 79% of animals developed PTSD and 21% were resistant. RT-qPCR analysis showed a differential increase in LINE-1 expression between hippocampus and cortex in resistant animals compared to control and susceptible, while bisulfite sequencing of LINE-1 promoter revealed changes on CpG and non-CpG methylation among the groups. Our findings suggest that LINE-1 expression influences the development of PTSD, opening the possibility to use it as therapeutic target to prevent or treat this neuropsychiatric disorder.

Keywords: LINE, 1, PTSD

*Speaker

Profile and dynamics of LINE-1 RNA and ORF1p expression in the aged and diseased brain

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Aging and the dysregulation of transposable elements are two closely linked processes, particularly characterized by the derepression of LINE-1 retrotransposons. On the one hand, aging is marked by LINE-1 derepression, and on the other hand, LINE-1 derepression leads to genomic instabilities, epigenetic alterations and inflammation which are hallmarks of aging and neurodegeneration. LINE-1 activation could therefore be involved in the pathogenesis of age-related neurodegenerative diseases. However, our knowledge of the expression and localization of LINE-1-encoded proteins in the central nervous system is limited. This study investigates, in the mouse and human brain, the expression and interactions of LINE-1 and its encoded protein ORF1p, a chaperone and RNA-binding protein. By establishing a novel approach combining brain mapping with deep-learning algorithms for cell segmentation on large-scale pyramidal images, we characterized ORF1p expression in the murine brain under physiological conditions. ORF1p is expressed in $\approx 20\%$ of murine brain cells and is neuron-specific. In aged mice, ORF1p expression in neurons increased in several brain regions including the midbrain and striatum to up to 30%. The transcriptomic analysis of TE expression in human post-mortem dopaminergic neurons revealed an increase in full-length LINE-1 expression in aged compared to younger neurons. Dysregulation of TE transcripts was also observed in post-mortem tissues from individuals affected by Alzheimer or Parkinson disease compared to controls. In mouse brain and human dopaminergic neurons in culture, ORF1p interacts with proteins related to mRNA splicing, ribosome biogenesis and nuclear proteins involved in nuclear envelope biology and transcription, which were modified during in vitro stress. This work contributes to a better understanding of the extent of ORF1p protein and LINE-1 expression in the brain under pathophysiological and aging conditions, strengthens the hypothesis that LINE-1 activation is linked with brain aging and opens the way to decrypt ORF1p biology.

Keywords: LINE, 1, ORF1p, aging, neurons, brain

*Speaker

Modeling the impact of L1 expression on colorectal cancer progression

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Colorectal cancer (CRC) stands as the third most prevalent cancer type, contributing significantly to cancer-related deaths. CRC develops through the sequential acquisition of somatic mutations in APC, KRAS and TP53 leading benign tumors to evolve into aggressive malignancies. Most CRCs follow the chromosomal instability (CIN) pathway, characterized by an increased rate of chromosomal rearrangements, although the molecular bases are not well understood. Long interspersed element-1 (L1) is a type of transposon capable of causing insertional mutagenesis and DNA damage. Elevated L1 activity has been associated with CRC, contributing to genomic instability and serving as a potential biomarker. However, the functional contributions of L1 in CRC, particularly during the early stages of tumorigenesis remain poorly understood, mostly because experimental models studying L1 function are scarce or nonexistent. Thus, there is a need to define the timing and mechanisms by which retrotransposition influences the early steps of CRC. To pursue this goal, I am establishing 2D and 3D models representing colorectal cancer initiation and progression to manipulate L1 expression and determine its function in CRC biology. Elucidating the functional role of L1 in CRC initiation and progression will provide valuable insights into the molecular mechanisms underlying CRC development. Understanding the interplay between L1 and key genetic events in CRC could potentially open new avenues for diagnostic and therapeutic strategies in combating colorectal cancer.

Keywords: L1, colorectal cancer, chromosomal instability, APC, TP53, KRAS, organoids

*Speaker

atena: an R/Bioconductor package for the analysis of transposable elements

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The quantification of RNA expression of transposable elements (TEs) requires specialized software and annotations outside the standardised pipelines and data sources employed in the analysis of RNA sequencing (RNA-seq) data. This often puts a burden on the users of such software, who first need to pull and combine input annotations from heterogeneous sources and formats and, second, parse the output quantifications before they can be fed into the next tool for a downstream analysis, such as a differential expression. The Bioconductor project is an open-source software repository widely adopted for the analysis of RNA-seq data. Bioconductor facilitates the interoperability of its software packages by defining a core set of data containers and interfaces to annotation databases. Here we present atena, a Bioconductor package that provides, in the first place, efficient and accurate re-implementations in R of three of the most popular methods for TE expression quantification: TEtranscripts (Jin et al., 2015), ERVmap (Tokuyama et al., 2018) and Telescope (Bendall et al., 2019). In the second place, atena also provides a single interface to download and flexibly parse into TE annotations all the Repeat-Masker track data available at the UCSC Genome Browser using different algorithms, including a re-implementation in R of the one by Bailly-Bechet et al. (2014). Furthermore, it provides a fourth expression quantification method, called atena, which is built upon the other three to address some of their shortcomings. We have used atena to investigate the contribution of TEs to the postnatal changes in expression following a fetal inflammatory response in extremely preterm neonates, using the newborn screening RNA-seq data produced by Costa et al. (2021). The atena package is publicly available at https://bioconductor.org/packages/atena.

Keywords: annotation, quantification, software, fetal inflammatory response

*Speaker

Investigating TE distribution across replication timing programmes in early mouse development

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Preimplantation development is a crucial period marked by intricate genomic processes, including the emergence of DNA replication timing (RT) and the transcriptional activation of transposable elements (TEs). TEs are ubiquitous DNA elements within eukaryotic genomes with the potential to influence gene expression and genome stability. However, the crosstalk between TEs and host DNA replication is poorly understood. Here, in this study, leveraging computational genomic analysis on single cell repli-seq datasets from our laboratory, we systematically examine the genomic landscape of TEs in relation to RT in mouse preimplantation embryos. We observe a heterogenous distribution of TE across the different RT zones and reveal a different distribution pattern of TEs according to their evolutionary age. Our study suggests a potential regulatory role of TE in DNA replication dynamics during mouse preimplantation development.

Keywords: Transposable element, replication timing, genetic distribution, mouse embryo

*Speaker

Epigenetic transposon regulation in a 3D cellular model of MORC2-associated neurodevelopmental disorders

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Mutations in epigenetic or chromatin regulators are a frequent cause of neurodevelopmental disorders. MORC2 is a chromatin modifying ATPase in which missense mutations lead to syndromes diagnosed as Charcot-Marie-Tooth disease and spinal muscular atrophy. At a molecular level, MORC2 is associated with human silencing hub (HUSH) complex in epigenetic silencing of transgenes and repetitive genetic elements including L1 retrotransposons and protocadherin genes. However, whether and how these functions are perturbed in MORC2 neurodevelopmental disorders is largely unknown. In this study we use a 3D organoid model to study the role of MORC2-mediated gene and transposon silencing in human brain development. CRISPR gene-editing was used in human induced pluripotent stem cells (hiPSCs) to generate clones bearing two neurodevelopmental disorder disease mutations (E27K, S87L) in the *MORC2* gene. Undirected cerebral organoids were generated from each hiPSC line. To show how these mutations influence early neural development; transcriptomic, epigenomic and morphological analysis were performed at different time points of organoid culture. Interestingly, we observed distinct molecular phenotypes of repeat element deregulation in the two disease mutants, in line with previous data suggesting that loss- and gain-of-function effects may arise from different disease genotypes. Our findings provide a molecular and functional basis for understanding MORC2 neurodevelopmental disorders and represent an example of repetitive element deregulation as a potential driver of neurological disease.

Keywords: Repetitive genetic elements, Epigenetic regulation, Neurodevelopmental disorders

*Speaker

Effect of weight loss on the methylation of human sperm transposable elements

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Humanity is heavier than ever. In 2023, nearly half of French adults were overweight or obese. A series of European surveys recently reported that almost one in three French adults admitted being on a diet, trying to lose weight. Resistance to diet-induced weight loss may be an evolutionary legacy, whereby our ancestral bodies were programmed to ensure enough energy storage for survival in hunger times. We hypothesize that weight loss leaves an epigenetic imprint that renders weight regain easier. While the epigenetics changes associated with high fat-induced obesity have been extensively studied, the epigenome response to a weight loss is poorly described. Previous work from our group using short-read sequencing identified that, compared to lean men, spermatozoa from obese men carry a distinct epigenetic signature at genes controlling brain development and function. Profiling 5-methylcytosine mark revealed that a surgery-induced weight loss triggers an epigenetic remodeling at genes involved in the control of appetite. Reanalysis of the data suggest that some intergenic regions associated with TEs may also be modified (pilot data). Given the rapid increase in obesity prevalence across generations, we hypothesize that epigenetic remodeling at human sperm TEs participate in the acceleration of this pandemic over generations. Our preliminary results using long-read sequencing revealed a near-complete map of the human sperm Y chromosome, which controls spermatogenesis. This chromosome remained the most incomplete mapped so far because of its highly repetitive structure. Our pilot Nanopore sequencing support that we should be able to achieve an unprecedented read of DNA methylation at TEs in the human sperm epigenome. Our goal is to take advantage of this technology to demonstrate their direct implication in obesity. These hotspots may indeed represent an inherited testimony that can be transmitted to future generations to predispose their (epi)genetic make-up to easier weight gain or fat storage.

Keywords: Weight loss, obesity, spermatozoa, epigenetic inheritance, transposable elements, prognostic markers, long read sequencing

*Speaker

The role of LINE-1 elements in the induction of type I interferon following epigenetic dysregulation

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The impact of Transposable Elements (TEs) in health and disease has been cemented by mounting evidence. Among the multifaceted co-opted functions of these elements is their involvement in immunity. Here, following on from previous work where we showed that the loss of the epigenetic complex - the Human Silencing Hub (HUSH) – can lead to an interferon (IFN) cascade downstream of the dsRNA sensor MDA5, we sought to shed light on the role of Long INterspersed Elements-1 (LINE-1) in nucleic acid sensing. To this end we employed a short hairpin RNA, selected from a panel of shRNAs targeting LINE-1 subfamilies, which is effective at blocking the type I interferon response induced upon inactivation of the chromodomain-containing component of HUSH, MPP8. By performing total RNA-sequencing of IFN reporter cells expressing this hairpin together with shMPP8, we could computationally identify self-RNAs that are candidates for inducing a type I IFN response and which may be involved in autoimmunity. Thus, our results bring us one step closer to disentangling the intricate role of LINE1s in immunity.

Keywords: Epigenetics, Immunity, LINE1, HUSH

*Speaker

Retrotransposon activity predicts cancer therapy response

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Cancer therapy has progressed significantly in the past two decades, yielding revolutionary cancer treatments, including targeted therapies and immunotherapy. However, the reasons some patients respond to these treatments and others do not remain a mystery in the field. Recent studies have shown that the immune system may play a role in response to targeted therapies against EGFR- and ALK-driven tumors. The marked importance of the immune system for treatment response to targeted therapies indicates a need to monitor tumor immunogenicity to better predict response. Here, we find that retrotransposon activity could predict response to therapy. In syngeneic, orthotopic murine models of ALK-driven lung cancer, we find a treatment responsive tumor (EA2) to have increased retrotransposon expression compared to tumors that have residual disease post-treatment (EA1 and EA3). The increased retrotransposon expression is observed along with an increased expression of interferon genes in EA2 but not EA1/3, combined with the known immune infiltration in EA2, point to the possibility that retrotransposon expression is driving immune states that favor response to the targeted therapy alectinib. We are developing plasma cfDNA-based biomarkers to capture these altered retrotransposon states to better predict response to targeted therapy. In summary, this study aims to develop more informative biomarkers, leveraging often neglected genomic sequences, to enhance our understanding of the biological underpinnings of treatment outcomes, ultimately contributing to the development of personalized and more effective cancer therapies.

Keywords: cancer, immunotherapy, targeted therapy, EML4, ALK, immunogenicity, retrotransposons, cfDNA

*Speaker

Understanding the role of L1 retrotransposons in the context of dopaminergic neuropathology

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The retrotransposon long interspersed element-1 (L1) occupies nearly 20% of the human genome. Beyond their proven capacity for germline retrotransposition, primate and rodent L1 sequences are expressed and can mobilise in certain somatic cells, including the neuronal lineage. However, the contributions made by L1 to neuronal physiology and neurological disease are largely unknown. In this study, we are investigating L1 activity in substantia nigra (SN) dopaminergic (DA) neurons - a neuronal subset whose selective degeneration constitutes the hallmark of Parkinson's disease (PD). To determine whether L1 contributes to DA neuron vulnerability during neurodegeneration, we have assayed L1 expression in DA neurons under normal and stress-induced conditions in vitro and in vivo.
Our data show that L1 mRNA and proteins are expressed in mouse and human DA neurons. In adult mouse midbrain, L1 mRNA expression is higher in SN compared to ventral tegmental area DA neurons, and this difference is already apparent during midbrain development. Using a common PD experimental model where neurotoxic stress is induced by 6-hydroxydopamine (6-OHDA) injection in the mouse midbrain, we observe significant DA neuron loss without notable differences in L1 mRNA or protein expression. By contrast, when we examine post-mortem human midbrain tissue sections, we find L1 ORF1p expression is lower in the remaining DA neurons from PD patients compared to age-matched controls. To better dissect the association and temporal dynamics of L1 expression and DA neuron toxicity, we established a novel in vitro model of human induced pluripotent stem cell derived-DA (hiPSC-DA) neurons. Exposure to subtoxic levels of 6-OHDA significantly increases ORF1p expression in hiPSC-DA neurons. This could suggest that high L1 expressing-DA neurons are selectively lost during PD progression.
Despite contrasting responses in mouse and human models, L1 expression dynamics throughout disease progression emerge as a potential hallmark of DA neuropathology, warranting further investigation.

Keywords: LINE 1, Dopaminergic Neurons, Parkinson's Disease, Neurodegeneration

*Speaker

The role of transposable elements in pregnancy complications

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Complications of pregnancy, such as recurrent pregnancy loss, pre-eclampsia, fetal growth restriction, and spontaneous preterm birth, affect _˜20 % of human pregnancies, causing maternal and fetal morbidity and mortality. Although the molecular aetiology of these disorders is not well understood, they are thought to share a common pathogenesis in insufficient uterine invasion by the placenta. Transposable elements, through their capacity to quickly generate genetic variation and influence host gene regulation, may contribute to species-specific placental gene expression, and processes such as placental invasion. Previously, we have shown that multiple endogenous retrovirus (ERV) families exhibit regulatory potential in placenta. These largely primate-specific elements are bound by transcription factors with key roles in placental development, and we used CRISPR-Cas9 excisions to show that several ERVs act as enhancers for genes that are important for placental function, such as CSF1R, ENG and PSG5. Research is now underway to explore whether human genetic and epigenetic variation at ERVs may contribute to pregnancy complications, using samples of placenta from normal and complicated pregnancies, and human trophoblast organoids.

Keywords: Endogenous retrovirus, placenta, pregnancy complications, pre, eclampsia, LTR10A

*Speaker

DNA virus infections shape transposable element activity in vitro and in vivo

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Transposable elements (TEs) have been shown to be activated in the context of viral infections, but the mechanisms and functional consequences of this induction are not understood. Here, we show strong activation of TEs in the context of DNA virus infection and investigate the molecular mechanisms of how TEs are induced. We demonstrate that herpesvirus infection leads to a robust expression of the MLT and THE1-class of LTR containing retrotransposons as well as a subset of long-interspersed nuclear elements-1 (LINEs), Alu-elements and some HERVs. Mechanistically we demonstrate that this TEs upregulation is induced by two pathways that act synergistically: de-repression of KAP1/TRIM28 mediated by phosphorylation and expression of the pioneer factor double-homeobox 4 (DUX4). DUX4 is known to be crucial for TEs induction during zygotic genome activation in early embryonic development. In adults, DUX4 is usually silenced and we previously showed that DUX4 expression is induced by infection with various DNA viruses. We demonstrate binding of DUX4 to TEs upon herpesviral infection and analysis of genes adjacent to TEs shows pathways that are known to be crucial for tumor development. Overexpression of DUX4 significantly induced TEs expression, while its knockout (KO) diminished TEs expression upon HSV-1 infection, underscoring the essential role of DUX4 in TEs activation. Interestingly, analysis of single cell sequencing data from patients with DNA virus infection showed that expression of TEs is also of relevance in vivo, especially in tumors that are caused by oncogenic DNA viruses.
Taken together, our data show how TEs are induced by DNA viruses, in particular oncogenic viruses of the herpesviridae, papillomaviridae and polyomaviridae families. TEs expression is known to be a hallmark of oncogenesis, and therefore it is tempting to speculate that viral induction of TEs contributes to viral oncogenesis.

Keywords: Transposable Elements, DNA virus, Herpesvirus, HSV, 1, HCMV, KAP1, TRIM28, DUX4, LINE, 1, LTR

*Speaker

Role of Transposable elements in the transcriptional programming of CD4 T lymphocytes

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The immune system is efficient at protecting the host against a wide diversity of dangers (tumors, viruses, bacteria and parasites). Its efficacy lies in its adaptability, allowing it to tailor responses based on the nature of the danger. CD4 T lymphocytes, which orchestrate immune responses, exhibit remarkable functional plasticity; according to environmental cues, they activate distinct gene networks adjusting their phenotype and functions. Consequently, CD4 T cells can differentiate into diverse T helper (Th) cell subsets, crucial for effective immune responses. Our recent findings demonstrate that in Th2 cells, transcriptional specificity is largely determined by a restricted set of endogenous retroviruses that have been exapted into cis-regulatory activator modules. Expanding on this, our study delves into the broader impact of transposable elements (TEs) on genomic architecture shaping T cell identity. Integrating transcriptomic and epigenetic analyses, we find that TE-derived sequences contribute significantly to the coordination of CD4 T cell programming. Notably, in Th1 cells, pivotal for host defense against cancer or viral infections, a substantial fraction of Tbet binding sites, master regulator of the lineage, originates from LINE2 TEs. These TEs likely integrated into the genome before mammalian radiation, In the course of evolution, the 5' region of LINE2 has degenerated, yet the Tbet motif persists within CD4 enhancers adjacent to vital immune genes, offering advantageous properties to the cell.

Furthermore, TE-mediated recruitment of Kruppel-associated box zinc finger proteins (KZFPs) appears to repress enhancers associated with alternative cell fates, maintaining Th cell lineage stability. Different TE families seem to restrict T cell plasticity by recruiting transcriptional repressors, thereby reinforcing specific lineage programs.
In summary, our data underscore the evolutionary co-option of certain transposable elements in shaping and perpetuating T cell integrity, both by the introduction of immune TFBS in the T(h)-cis regulatory elements and controling their epigenetic state and function.

Keywords: TE, Immunity, T CD4, cell fate & plasticity, KZFP, LINE2

*Speaker

Non-invasive multi-cancer detection targeting DNA hypomethylation of LINE-1 retrotransposons

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Circulating tumor DNA (ctDNA) released by tumor cells into the blood stream bears all the molecular alterations of the tumor of origin, enabling non-invasive molecular profiling and disease monitoring. However, targeting small fractions of tumor DNA when the tumor burden is low remains one of the biggest challenges of liquid biopsy. In addition, targeting mutations that may have less than one alteration copy per milliliter of plasma further complicates the chances of detection.

Therefore, we have developed a new highly sensitive strategy for detecting cancer-specific signatures in blood based on the methylation patterns of repeat sequences. We have centered our study on primate specific Long Interspersed Nuclear Element-1 family (L1PA and L1HS) which are known to be hypomethylated in multiple types of cancers. Firstly, we have designed a PCR-based bisulfite deep sequencing, targeting 35 CG sites. We then developed a prediction model integrating methylation haplotypes at the single molecule level. Resulting machine learning-based classifiers showed promising classification rates in 6 types of cancer to discriminate healthy from tumor plasmas.

We now want to further increase the detection sensitivity and the universality of our approach, by maximizing the number of CG sites targeted notably. To do so, we are transposing the method to a capture-based targeting of LINE-1 elements. Our preliminary results show that we are able to hit more LINE-1 copies with high specificity (89% on-target rate). Moreover, we obtain methylation information for healthy donors along the 95 L1HS CG sites. Based on these preliminary results, this approach could lead to the development of more efficient, non-invasive diagnostic tests that can be applied to multiple types of cancer.

Keywords: LINE, 1 elements, circulating DNA, DNA methylation, targeted sequencing, hybridization capture, multi, cancer detection, machine learning

*Speaker

Harnessing ERV Expression to Ignite Anti-Tumor Immunity

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Endogenous retroviruses (ERVs), integrated remnants of ancestral viral infections, are largely epigenetically silenced in the human genome to circumvent potential harm. Nonetheless, accumulating evidence now posits the de-repression of ERVs as a potential weapon against cancer. This is based on the ability of ERV-derived elements to stimulate antiviral signalling, and consequently drive immune responses. Here, I will highlight the role of TRIM28, a pivotal regulator of ERV silencing, as a potential target for cancer treatment. Our approach utilizes TRIM28 genetic silencing techniques alongside TAK-981, an innovative SUMOylation inhibitor that blocks TRIM28's SUMOylation. The SUMOylation of TRIM28 is a critical mechanism involved in the recruitment of epigenetic silencing complexes that inhibit ERVs. Overall, I will present the possibility of targeting TRIM28 to derepress ERV expression and trigger ERV mediated activation of IFN/NF-B signalling and necroptosis in the cancer setting.

Keywords: ERVs, immunogenic cell death, innate immune signaling, Z, RNA, cancer

*Speaker

Investigating the Role of ERV-Derived Transcripts in Amyotrophic Lateral Sclerosis Pathogenesis

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TDP-43 proteinopathies are a group of diseases that include amyotrophic lateral sclerosis (ALS). TDP-43 proteinopathies are defined by loss-of-function of DNA- and RNA binding protein, TDP-43. Investigation into TDP-43 has uncovered potential mechanisms of TDP-43 proteinopathy pathogenesis. TDP-43 has been shown to bind to endogenous retrovirus (ERV) sequences in the human genome, and both TDP-43 knockdown and overexpression promote ERV dysregulation in vitro and in vivo. ERVs offer new perspective on genomic dysregulation and disease. Transposable elements – artifacts of viral integrations spanning hundreds of millions of years of evolution – make up 45% of the human genome. 8% of the human genome, ERVs are the most recent retrotransposon insertions, the youngest integration occurring $<$ 6M years ago. ERV loci exhibit polymorphism across human populations due to replication and recombination, thus contributing to genomic diversity. Young ERVs contain intact proviral elements, i.e. LTRs, gag, pol, and env open reading frames, which may dysregulate gene expression and initiate inflammation in disease. Current tools fail to quantify ERV locus expression and define ERV transcript structure. Short-read RNA-sequencing (RNA-seq) is a technology capable of mapping unique 200bp reads to the genome with high accuracy. However, short-read RNA-seq is for ERVs is flawed due to their repetitive genomic content and large average size (7kb). Conflicting reports on ERV dysregulation in ALS between annotations and algorithms highlight the failures of current tools to 1) reliably map ERV transcripts to their loci and 2) denote ERV transcript structure. Long-read RNA-seq offers a solution for these limitations by generating reads up to 10kb long, thereby increasing ERV transcript mapability. Using long-read RNA-seq, I will be able to analyze ERV expression in the transcriptome with high confidence and at a higher resolution than previously possible. I propose to use whole transcriptome long-read RNA-seq to profile ERV splicing behavior in TDP-43 proteinopathies.

Keywords: Endogenous retroviruses, ERVs, TDP, 43, Amyotrophic lateral sclerosis, ALS, long, read RNA, seq

*Speaker

An atlas of Alu exons across human tissues

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With more than 1 million insertions in the human genome, Alu elements have and continue to shape primate genome architecture. One mechanism by which Alu elements contribute to genome evolution is through the formation of novel exons in mRNA transcripts. Such Alu exonization may represent an evolutionary strategy by which host genomes can modulate protein expression and protein behavior. Recent advances in long-read sequencing technology support more comprehensive detection and quantification of TEs. We have developed ESPRESSO-TEA (Error Statistics Promoted Evaluator of Splice Site Options – Transposable Element Analysis), a computational pipeline to profile locus-specific TE expression from long-read RNA-seq. ESPRESSO-TEA characterizes TE-derived exons within full-length transcripts, thereby capturing the complex, spliced structures of different TE-containing transcript isoforms. Using this platform, we characterize the transcriptional landscape of Alu exons across 30 human tissues. We highlight mechanisms by which Alus may drive tissue-specific and species-specific differences in gene expression and protein production. We expect our research will shed light on evolutionary and functional innovations that distinguish human and non-human primates.

Keywords: Alu, exonization, long, read RNA, seq

*Speaker

Role of histone acetylation in the regulation of transposable elements in trophoblast cells

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The placenta is one of the most enigmatic and unique organs. Acting as the interface between multiple genetically different individuals, it is crucial to maintain the homeostasis of both and to allow the development of the foetus. Despite convergently evolving across multiple species, the diversity results in a lack of a translatable animal model. The study of the placenta is further complicated by the crucial role that transposable elements (TEs) play during its differentiation, with some producing proteins necessary for the organ's growth: for example, Syncytin-1. Each species boasts its own unique fingerprint of TEs and, therefore, a consequent transcriptome during placental development. Emphasising the lack of a translatable model. To address this critical gap in the field, we currently utilise a cellular model by differentiation of human embryonic stem cells to trophoblast stem cells. Using this model system, we are investigating how the histone acetylation pathways impact the expression of transposable elements during placental development. Utilising this system, we are aiming to investigate how TEs are involved in both the healthy establishment and differentiation of the placenta, as well as to show how their aberrant expression is implicated in disease states such as pre-eclampsia.

Keywords: Epigenetics, histone, trophoblasts placenta, pre, eclampsia, cellular model, in vitro, pregnancy, loci, subfamily

*Speaker

Investigating the intersection of LINE-1 retrotransposition and DNA replication

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*L*ong *in*terspersed *e*lement 1 (LINE-1, L1) is the only active retrotransposon in modern humans and its aberrant overexpression is found in nearly half of human cancers. L1 generates *de novo* insertions of itself via a 'copy-and-paste' mechanism that is dependent on two protein-coding open reading frames (ORFs): ORF1p, an RNA-binding protein and ORF2p, an endonuclease (EN) and reverse transcriptase (RT). Accumulating evidence implies that the insertion of L1 into the genome occurs during S phase and that DNA replication and replication-coupled repair factors limit retrotransposition. Additionally, L1 expression generates molecular vulnerabilities to the loss of DNA replication and repair factors. These synthetic lethal interactions suggest a model wherein L1 retrotransposition intermediates hinder DNA replication progression, a condition referred to as DNA replication stress (RS), that can lead to fork collapse, DNA damage, and consequent genomic instability. To explore this model and impact of L1 expression on replication fork dynamics, we performed DNA fiber assays and found L1-expressing cells display a marked reduction in replication fork velocity accompanied with an asymmetric pattern of sister replication tracts, an indication of direct fork stalling. Intriguingly, we observed higher frequency of newly fired DNA replication origins in L1-expressing cells. We found that inhibition of new origin firing using CDC7 inhibitors completely rescued L1-induced RS, suggesting its dependence on aberrant origin. Mitotic entry with persistent replication intermediates through S/G2 checkpoint triggers a mitotic DNA synthesis (MiDAS) and compromises chromosome segregation, causing chromosomal breakage and genome instability. Indeed, we found higher frequencies of MiDAS and cytological markers of chromosome missegregation upon L1 expression. We are currently testing the hypothesis that aberrant origin firing underlies L1-induced replication stress and chromosome instability. Our findings are expected to reveal the molecular mechanisms underlying L1-associated DNA damage and genome instability, a hallmark of cancers.

Keywords: Retrotransposition, DNA replication stres, DNA repair, genome instability, LINE, 1

*Speaker

The HTLV-1c genomic graveyard reveals host-virus interactions

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Retroviruses infect a range of vertebrate hosts. Upon infection, the viral genome is reverse transcribed and integrated into the host genome. The integrated provirus and host genome are enmeshed and have an ongoing reciprocal influence on one another, impacting both host and viral fitness. The integrated provirus thus has the potential to archive host-virus interactions.
Human T-cell leukemia virus 1 (HTLV-1) is a pathogenic retrovirus, which upon infection integrates its 9kb genome into the human genome. _˜10-20 million people carry HTLV-1 worldwide. Central Australia has the highest rate of HTLV-1 infection globally, with a prevalence of _˜40% reported in some remote Communities. HTLV-1c is the highly divergent molecular variant of HTLV-1 found in Australia.

HTLV-1 integration into the human genome has profound consequences on its target cell. However, the consequence of integration on HTLV-1 fitness is poorly understood. To characterise the genomic interface of human-HTLV-1c interactions, we have performed end-to-end sequencing of $>$ 400 individual HTLV-1c genomes using the Oxford Nanopore platform. Samples were obtained from patients with bronchiectasis (n=3), asymptomatic carriers (n=3), and humanised mice in the late stage of HTLV-1c infection (n=2).
The HTLV-1c landscape is overwhelmingly dominated by defective clones. HTLV-1c provirus is highly enriched in CCR4+ cells, which contain $>$ 90% defective provirus. Most defects disrupt the canonical retroviral genes, consistent with clonal expansion seen in disease. Unexpectedly, a large subset of proviral integrants contain internal human genomic sequences, including exons and repetitive elements, potentially placing these sequences under the control of strong LTR promoters. Breakpoint analyses revealed that in-del junctions have homology to the HTLV-1c LTR, suggesting a recombination-based mechanism of deletion. CCR4+CD4+ T-cells are thought to drive HTLV-1 associated disease. We found CCR4-CD4+ T-cells enriched for full-length HTLV-1c provirus, raising the possibility that CCR4-CD4+ T-cells could provide a reservoir of full-length, replication-competent provirus.

Keywords: bronchiectasis, infectious mobile DNA, retrovirus

*Speaker

Misdirected activity of antiviral proteins against syncytins

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Syncytin-1 and syncytin-2 are envelope glycoproteins encoded by human endogenous retroviruses that have been exapted for the fusion of cytotrophoblast cells into syncytiotrophoblasts during placental development. Interestingly, pregnancy complications have been associated with altered expression of interferon-stimulated genes, including antiviral restriction factors. We therefore hypothesize that antiviral proteins may mis-target syncytins and interfere with normal placenta formation when aberrantly active during pregnancy.
In an initial screening, we tested a panel of restriction factors to determine which antiviral host proteins might inhibit syncytin-mediated fusion. In agreement with a previous study (Buchrieser et al., 2019), we found that the antiviral protein IFITM3 inhibits syncytin-1-mediated membrane fusion. Moreover, guanylate binding protein 2 and 5 (GBP2 and GBP5) suppressed fusion mediated by both syncytins, although syncytin-1 and -2 differed in their sensitivity. Furthermore, expression of GBP2 and GBP5 is interferon-inducible and driven by solo-LTRs of the ERV9 family (Srinivasachar Badarinarayan et al., 2020). In an earlier study (Braun et al., 2019), our group had shown that GBPs prevent the proteolytic maturation of viral envelope proteins by inhibiting the activity of furin. Surprisingly, we did not observe GBP-mediated suppression of syncytin-2 cleavage, whereas syncytin-1 maturation was inhibited by both GBPs. Our investigation further revealed that syncytin-2 is efficiently cleaved by PCSK7, a protease from the same protein family as furin. In contrast to furin, the proteolytic activity of PCSK7 was not inhibited by GBPs.
Overall, this study revealed that aberrant expression of antiviral factors can reduce syncytin-mediated fusion. However, syncytin-1 and syncytin-2 can be processed by several host proteases, which may partially compensate for impaired cleavage in the presence of GBPs. Altogether, our results provide insights into pathological placenta formation as a result of innate immune responses that are misdirected against endogenous retroviral proteins.

Keywords: endogenous retrovirus, syncytin, cell fusion, antiviral proteins, guanylate binding proteins

*Speaker

The role of TP53 on transposable elements in paediatric cancer

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Background: Transposable elements (TEs) are dynamic repetitive regions which generate mutations and structural variants. TP53 plays a crucial role in suppressing TE movement to maintain genomic stability. The relationship between *TP53* and TEs has been extensively studied in tumours, but not the germline. Individuals with germline *TP53* pathogenic variants have Li-Fraumeni Syndrome (LFS), a cancer predisposition syndrome with a high lifetime risk of cancer in various tissues. This study aims to characterize the TE landscape in individuals with Li-Fraumeni Syndrome and determine how this may contribute to increased cancer risk.
Methods: MELT and xTea were used to identify TEs in young adults with (n=64) or without (n=79) a germline *TP53* variant. TE calls were merged with SURVIVOR and variants were annotated with AnnotSV. To assess the influence of *TP53* on TE location, we quantified TEs across genomic windows and identified the top 200 significantly different regions with the Mann-Whitney U test, adjusting for multiple comparisons. We used these regions to develop a gradient-boosted tree model with 5-fold cross-validation to predict *TP53* status.

Results: Individuals with germline *TP53* variants harboured significantly fewer ALU and LINE1 elements in their germline genome compared to the control dataset ($p < 0.001$). The phenomenon was consistent across chromosomes and significant in chromosomes 5 and 11 (FDR< 0.05). A gradient-boosted tree model was able to differentiate patients with and without a germline *TP53* variant with an AUC of 0.7 on the unseen test set.
Conclusion: Germline *TP53* variants may effect the frequency and location of TE insertions, which may influence nearby genomic variations and lead to cancer development. Analyzing TEs in individuals with LFS will enhance our understanding of accelerated cancer development in these patients, informing future research for diagnostic and therapeutic approaches.

Keywords: cancer, genomics, machine learning, li fraumeni syndrome, cancer predisposition, paediatric

*Speaker

Alu Elements and Structural Variants in BRCA1: Unveiling Genetic Puzzles in Korean Women's Breast Cancer

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Breast cancer is a common cancer in women around the world, and the incidence of breast cancer in women under the age of 50 is increasing recently. In addition, breast cancer is susceptible to hereditary factors, including family history, so it is important to identify and diagnose a patient's genetic variants. Therefore, this study aims to deepen our understanding of the genetic basis of breast cancer by identifying structural variants (SVs) induced by the *Alu* element in the *BRCA1* gene. We utilized the high-throughput capabilities of the PacBio platform for long-read sequencing and Twist's target enrichment method to investigate *Alu* element-based SVs in the *BRCA1* gene in 51 tissue samples (29 breast cancer tissues and 21 normal tissues) from Korean female breast cancer patients. Comprehensive analysis using SMRT Link software and third-party tools were used to uncover the presence and pattern of SVs. We identified a total of 55 large and small genetic pockmarks in the BRCA1 gene and interestingly, 41 of these events were caused by *Alu* elements. The insertion of LTR12C upstream of the BRCA1 gene was found to be common in cancerous tissues of 10 breast cancer patients and 5 normal tissues. By analyzing the impact of *Alu* element variants in the *BRCA1* gene on the genetic causes of breast cancer, this study will contribute to the improvement of breast cancer diagnosis accuracy and the development of personalized treatment strategies. We expect that our results will play an important role in reducing mortality of breast cancer patients and provide new directions for breast cancer research.

Keywords: Alu element, structural variants, breast cancer, BRCA1

*Speaker

No Apparent Higher Frequency of Copy Number Alterations of the HERV-W_Xq22.3 Locus Region in Patients with Multiple Sclerosis

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Approximately 8% of the human genome are of retroviral origin. Human endogenous retroviruses (HERVs) derive from germ-line colonizations by distinct exogenous retroviruses that occurred millions of years ago. Few HERVs still encode retroviral proteins and HERV proteins have been involved in human physiology and pathology, including a pathophysiological role of multi-copy HERV-W, specifically HERV-W Envelope (Env) protein in Multiple Sclerosis (MS). The precise genetic origin of pathophysiologically relevant HERV-W Env protein remains unclear. A HERV-W Env-encoding locus (ERVW-1) encodes a full-length Env (Syncytin-1) that is involved in formation of the placental syncytiotrophoblast layer. Our own previous findings imply that, rather than ERVW-1, a HERV-W locus located in human chromosome Xq22.3 (HERV-W_Xq22.3) might be of relevance in MS. HERV-W_Xq22.3 lacks most of the proviral 5' portions, yet harbours a 542 aa env ORF interrupted only by a stop mutation at codon 39, the next downstream start located at codon 68. HERV-W_Xq22.3 Env is detected by HERV-W Env-specific antibodies. Mouse mAb GN-mAB_03 (3B2H4), employed for immunohistochemical detection of HERV-W Env in MS lesions, recognizes HERV-W_Xq22.3 Env, but not Syncytin-1, thus the protein detected by GN-mAB_03 in MS lesions might originate from HERV-W_Xq22.3. HERV-W_Xq22.3 locates within a genome region with copy number variations (CNVs). HERV-W_Xq22.3 within a CNV region is expected to alter the "normal", sex-specific number of HERV-W_Xq22.3 copies, potentially affecting expression levels of HERV-W_Xq22.3 accordingly. We studied a conceivable association of the copy number of the HERV-W_Xq22.3 locus region with MS by measuring copy numbers of the HERV-W_Xq22.3 locus region in gDNA from patients with MS compared to healthy controls. We did not detect significantly different copy numbers of the HERV-W_Xq22.3 locus region between those groups. Our results provide further insight into how the HERV-W_Xq22.3 locus may (not) be involved in the development of MS.

*Speaker

Keywords: human endogenous retrovirus, HERV, multiple sclerosis

Investigating the chromosomal instability signature of LINE-1 retrotransposition

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Long interspersed element-1 (LINE-1) is the only active, protein-coding transposon in humans that can self-propagate via RNA intermediates. LINE-1 overexpression and somatically-acquired LINE-1 copies are commonly detected in human cancers with *TP53* mutations. A recent pan-cancer analysis found associations between somatically-acquired LINE-1 insertions and chromosomal rearrangements, suggesting that LINE-1 retrotransposition activity may represent a major source of chromosomal instability in cancer genomes. To address this hypothesis, we have developed a Tet-On system to induce LINE-1 expression in *TP53* deficient retinal pigment epithelial (RPE-1) cells, which are nearly diploid cells. Using this system, we examined the genotoxicity and mutational consequences of LINE-1 expression in cells. Consistent with previous studies, we find LINE-1 expression robustly activates the DNA damage response, causes chromosomal breaks, results in micronuclei formation, and induces DNA replication stress. To assay the impact of LINE-1 expression on genome integrity, we performed whole genome sequencing (WGS) of single cells or clonal outgrowths to comprehensively assay the genomic alterations generated after induction of LINE-1 expression. We have found that single cells or clonal outgrowths induced with LINE-1 expression contain one or multiple *de novo* DNA copy-number alterations, including copy-number losses and gains, and whole chromosome losses. We have also detected a variety of short and long-range genomic rearrangements with one or multiple breakpoints attributed to LINE-1-encoded ORF2p endonuclease, demonstrating a direct role of LINE-1 in creating large structural rearrangements. Strikingly, we have found that cells exposed to LINE-1 expression also contain complex chromosomal rearrangements such as chromothriptic chromosomes, suggesting that LINE-1 expression can elicit chromothripsis. These studies highlight the scope of LINE-1-mediated genome instability, providing insights into how LINE-1 deregulation in malignancies may broadly contribute to cancer genome evolution via chromosomal instability.

Keywords: LINE, 1, chromosomal instability, DNA damage

*Speaker

Contribution of transposable elements in the sex gap longevity of different Drosophila species

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In Drosophila, like in many other animal species, females tend to live longer than males, a phenomenon known as sex gap in longevity (SGL). One of the possible causes underlying this phenomenon could be related to the high number of transposable elements (TE) in the Y chromosome (toxic Y effect). TE activity is normally repressed by epigenetic mechanisms. However, it is known that this regulation is disrupted with age. Since the Y chromosome is rich in TEs, more TEs may become active in old males than in old females, generating more somatic mutations, and reducing longevity in males. In this work, we studied the natural variation in SGL in several natural populations of three different Drosophila species that vary in their TE content: *Drosophila melanogaster*, *Drosophila simulans*, and *Drosophila suzukii*. Furthermore, we found that the replacement of the Y chromosome between strains with different SGL reduces male lifespan over generations and thus increases SGL, suggesting an important role of the Y chromosome in male longevity. Finally, RNA-seq analysis from old and young flies suggested that there is an increased number of upregulated TE families in old samples, and more specifically in old males compared to old females, and that the total fraction of transcripts derived from repeats increase during aging depending on the species and the population tested. Overall, this work tries to better understand the genomic differences that lead to variation in longevity patterns between sexes, and emphasizes the importance of TEs in male longevity.

Keywords: Transposable Elements, longevity, natural populations, Y chromosome

*Speaker

Nanopore sequencing unveils structural features of somatic and germline retrotransposons

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Retrotransposons are the only type of transposable elements that remain active in human genomes. Although most elements are truncated, a few elements remain able to retrotranspose. In fact, somatic retrotransposition is a hallmark of various cancer types, with up to hundred insertions detected in colorectal cancers (CRCs).
Characterization of retrotransposon insertions has posed challenges with traditional short-read sequencing technologies. However, the emergence of long-read sequencing technologies has enabled the exploration of structural features of the insertions. Additionally, long-read sequencing offers the capability to identify retrotransposon insertions nested within other repetitive sequences. To systematically detect and annotate both somatic and germline retrotransposon insertions in 104 uterine leiomyomas and 62 CRCs, we developed a pipeline- Transposon De-

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tection in Oxford Nanopore Sequencing data (TraDetIONS). Using TraDetIONS, we identified 1495 somatic insertions in colorectal samples, however, uterine leiomyomas-benign neoplasms originating from mesenchymal tissue-exhibited no somatic insertions.
A comparative analysis of somatic and germline insertions unveiled disparities in terms of transposon classes, insertion length, and target site preferences. Furthermore, insertions featuring 5' inversion, processed pseudogenes, and nested retrotransposons-transposons nestled within other transposons-were detected. This analysis enabled the characterization of somatic and germline retrotransposition events, leveraging the long sequencing reads provided by Oxford Nanopore technologies.

Keywords: long, read sequencing, cancer, nanopore, colorectal cancer

H4K16ac mediated transposable over expression leads to inflammation in Preeclampsia

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Preeclampsia (PE) is a pregnancy-associated hypertension disorder which affects 5–10% of pregnant women each year worldwide. Here, we aimed to investigate the epigenetic mechanisms contributing to preeclampsia by genome-wide profiling of histone acetylations and transcriptome in healthy and preeclamptic placenta. We found a specific increase in acetylation level at histone H4 lysine 16 (H4K16ac) across transposable elements in preeclamptic placenta compared to control. H4K16ac hyperacetylation in pre-eclampsia is associated with increased transcript levels of endogenous retrovirus (ERV/LTR) and long interspersed nuclear element 1 (L1, LINE1) subfamilies of TEs in PE placenta from independent pregnancy cohorts. Furher, analysis of the additional cohorts alongwith this study, we discover upregulation of the LTR and L1 subfamilies as well as type I interferon (IFN) responsive genes upregulated in Preecalmpsia. These findings were consistent with the validation using RT-qPCR for the L1 ORF and 5' UTR as well as IFN-genes, indicating the contribution of H4K16ac-mediated upregulation TE contrbuting to inflammation in PE.

Keywords: H4K16ac, Preeclampsia, Transposable elements, Inflammation

*Speaker

Non-invasive multi-cancer diagnosis using DNA hypomethylation of LINE-1 retrotransposons

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The detection of circulating tumor DNA, which allows non-invasive tumor molecular profiling and disease follow-up, promises optimal and individualized management of patients with cancer. However, detecting small fractions of tumor DNA released when the tumor burden is reduced remains a challenge. We implemented a new highly sensitive strategy to detect base-pair resolution methylation patterns from plasma DNA and assessed the potential of hypomethylation of LINE-1 retrotransposons as a non-invasive multi-cancer detection biomarker. We have developed computational tools to accurately align sequencing data without a reference genome and applied prediction models, trained by machine learning algorithms, integrating patterns of methylation, overall and at the single molecule level. This assay, named DIAMOND (for **D**etection of Long **I**nterspersed Nuclear Element **A**ltered **M**ethylation **ON** plasma **D**NA), showed powerful correct classification rates discriminating healthy and tumor plasmas from 6 types of cancers, including 3 at localized stages, in two independent cohorts (AUC = 88% to 100%, N = 747). To push the DIAMOND assay towards a clinically applicable test, we also demonstrated that DIAMOND data can be used to perform copy number alterations analysis which improves cancer detection. We integrated this analysis in a classifier providing 'healthy' or 'cancer' labels for each sample and reached a detection of 91% of true positives for all cancers together on the independent validation cohort. This approach offers an optimized balance between the number of targeted regions and sequencing depth, which could extensively improve the sensitivity of ctDNA detection in a cost-effective manner and improve management of patients with cancer. This should lead to the development of more efficient non-invasive diagnostic tests adapted to all cancer patients, based on the universality of these factors.

Keywords: liquid biopsy, cancer, DNA, methylation, Aneuploidy, LINE, 1, biomarker, multi, cancer

*Speaker

Mapping transposable element activity in human brain development

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Transposable elements (TEs) have emerged as crucial regulatory elements, contributing to the rewiring of gene regulatory networks. Increasing attention is given to the role of TEs in the human brain, as it is the somatic organ with the most TE activity. Moreover, the underlying regulatory programs of human brain development are still not elucidated and the role of TEs herein is understudied. TEs exert a locus- and cell-type-specific function, but this is challenging to study because of their repetitive nature and the sparsity of single-cell sequencing data. In the past, most studies in this field focused on one brain developmental time point, one TE (sub)family, and one (bulk) omics layer at a time. This study aims to characterize TE-mediated gene regulation during human brain development at the single-cell level. Using TE-centric bioinformatics tools, we re-analyzed two time points (24 gestational weeks and three months postnatally) of a human post-mortem brain development dataset of single-cell RNA sequencing and ATAC sequencing. We discovered both expressed and chromatin-accessible TEs of different TE families, including LINEs, SINEs, LTRs, SVAs, and DNA transposons at these time points. Moreover, by doing differential expression and accessibility analysis, we found cell-type- and developmental stage-specific TEs. Finally, genes in the vicinity of these TEs were differentially expressed. Hence, we hypothesize that some of these TEs function as enhancers. In conclusion, we identified putative TE enhancers and transcripts, indicating a potential role in human brain development, which needs further experimental validation. Future research will focus on the regulatory dynamics of TE involvement across human brain development.

Keywords: Brain development, enhancers, single, cell sequencing, multi, omics, computational biology

*Speaker

NanoVar & NanoINSight: Characterization of genomic structural variants and transposable elements using low-depth nanopore sequencing

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Structural variants (SVs) are large genome alterations that are diverse in size and type including unbalanced, balanced, and complex variants. SVs are a major source of genetic diversity and many SVs are associated with Mendelian and complex diseases such as cancer and neurodevelopmental disorder. Our ability to properly characterize SVs was limited till the third-generation sequencing technologies were developed and significantly improved SV discovery despite the high sequencing error rate and low sequencing throughput. Transposable elements (TEs) are a subcategory of SV that play a crucial role in human evolution, genome instability, and disease development. Accurate characterization and mapping of TEs sequences is challenging due to their repetitive nature. Thus, short-read technologies fail to detect TE insertions. In 2020, our research group developed NanoVar, an optimized SV caller utilizing low-depth (8X) long-read sequencing data. NanoVar performs robustly in different genomic studies including mammalian and plant genomes. It outperformed other callers in genotyping accuracy and at low sequencing depths. Here, we present the latest release of NanoVar that includes a new feature called NanoINSight that allows characterization and comprehensive annotation of non-reference repetitive elements. NanoINSight workflow consists of utilizing the novel insertions' sequences extracted by NanoVar to generate multiple sequence alignments, from which consensus sequences are generated and then used as input for RepeatMasker to identify and classify repetitive elements. The updated version of NanoVar demonstrates faster and more precise performance compared with its earlier versions when tested with simulated and real datasets. We anticipate that accurate detection of SVs and novel insertions (TEs) by NanoVar/NanoINSight will contribute to a better understanding of diseases' genesis and development for ultimately improving diagnostic and therapeutic strategies.

Keywords: NanoVar, NanoINSight, structural variants, transposable elements, long, read sequencing, low, depth coverage

*Speaker

Storming TE-Storm: implications for short RNA-seq feasibility in transposon research examplified by ALS/FTLD (re)analysis

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Transposon transcriptional activity has been a suspected contributor to ALS/FTLD spectrum disorder since the 2010s (PMID:35415778), leading to the proposal of retrotransposon storm hypothesis of neurodegenerative diseases (PMID:29705598). TDP-43 and C9Orf72 pathologies were hypothesised as associated to TE overexpression (PMID: 22957047, 28637276). A number of studies failed to replicate these findings, while others reported discrepant TE subfamilies as hyperactivated (PMID:35415778). Most recent studies relied on short read RNA-seq and focused on the TE activity at the subfamily level, using inconsistent analysis methods.The latter may have contributed to the discrepancies between the results. Subfamily level analysis remains mainstream, as it theoretically overcomes mapping uncertainty for individual TE copies, however concerns were raised in the community (PMID:32576954 and 36338986).
We aimed at evaluating feasibility of TE analysis at the subfamily level (using synthetic and cell culture derived datasets) in RNA-seq datasets and to systematically analyse previously published and a new in-house human ALS/FTLD datasets to test the "storm" hypothesis and its association to TDP43 or C9Orf72 pathology.

Our simulation and investigation into iPSC transcriptomes demonstrated a high false positive rate for subfamilies analysis, which was driven by both mismapping between related but distinct subfamilies, and exaptation of TEs within longer transcripts, such as LINC00665. Our results suggest TE analysis at the subfamily level is unadvisable and may ultimately lead to the misinterpretation of transcriptomic changes.
Further analysis of ALS/FTLD datasets using a unified pipeline showed no consistent profile changes between different datasets (incl. for the previously suspected elements, e.g. HERVK), and no global transcriptional upregulation of TEs across datasets (equal numbers of up- and down-regulated loci in disease). We find further support for alternative gene isoforms as an explanation that leads to misidentification of TE activity change, illustrated through the STMN2 gene.

Keywords: neurodegeneration, neurodegenerative disease, ALS, FTLD, human disease, human transposons, human, RNA, seq, expression, transposon transcriptomics, transcriptomics

*Speaker

Co-opted endogenous retrovirus governed transcriptional and post-transcriptional regulation of Pregnancy Specific Glycoprotein 9 (PSG9) locus in the development of healthy and preeclamptic placentation

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Understanding the causes of the exceptional rate of evolution of the mammalian placenta is likely to aid the understanding of placental development and the aetiology of the human specific pregnancy disorder pre-eclampsia (PE). As a set of transposable elements (TE) are often lineage-specific and known to be co-opted for placental functioning, here we consider the TE binding partners of GATA3 and DLX5 that govern trophectoderm regulatory networks and have previously been shown to be grossly dysregulated in PE. This identifies a set of TEs, including the endogenous retroviral (ERV) LTR8B. LTR8B and another ERV, MER65, are enriched at the multigene pregnancy-specific glycoprotein (PSG) **gene array, a recently amplified, highly intra-specifically variable and dynamic primate specific genomic region. We find that both LTR8B and MER65 contribute to the evolution of the PSG genes and their functional diversification from the related membrane bound CEACAM gene family. MER65 elements promoted the initial evolution of secreted PSG variants by providing alternative polyA signal(s) of a truncated C terminus protein, ablating the transmembrane domain. By contrast, the LTR8B promoter/enhancer provides differential binding of transcription factors (TFs) (e.g. GATA2/3, DLX5, TFAP2A/C) and defines a diversified expression pattern of the PSG genes. CRISPR-Cas9 knockouts, expression rescue and 3D chromatin studies reveal that while LTR8B copies were co-amplified along with the PSG genes, PSG9 is exceptional. The LTR8B/PSG9 promoter/enhancer controls additional PSG members, trophoblast-specific TFs and several key pregnancy genes. This LTR8B/PSG9 governed regulatory network plays a central role in differentiation of the multinucleated syncytiotrophoblast. Dysregulation of GATA3 is associated with elevated levels of PSG9 in the maternal serum of PE patients in the first trimester, holding promise for the development of a predictive biomarker. We conclude that PSG9 represents an instance of taxonomically restricted TE recruitment for placental function with dysregulation associated with human-specific preeclampsia.**

Keywords: LTR8, Placenta, specific glycoproteins, Trophoblast development, Preeclampsia

*Speaker

Endogenous retrotransposon activity in the Drosophila intestine - towards the mechanisms of action of selfish DNA in the soma

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A growing body of research uncovers the importance of selfish DNA in somatic lineages throughout development and adult life. Endogenous retrotransposons, transposable elements that propagate via copy-and-paste mechanisms involving an RNA intermediate, occupy large portions of all eukaryotic genomes. A great majority of their multiple copies remains silenced in somatic cells, nevertheless, some are transcribed, and a small fraction retains its ability to mobilize, often in a tissue specific manner. Because of the highly repetitive nature of retrotransposons, identification of the precise active copies is often challenging. Consequently, the mechanisms that drive their somatic activity are not well understood.

In our previous work (Siudeja, van den Beek et al, 2021) we provided sequencing-based evidence of somatic retrotransposon mobility in the intestinal tissue of *Drosophila melanogaster*, which can lead to tumor suppressor inactivation and formation of gut neoplasia in aged midguts. Here, I will present our ongoing efforts towards revealing the mechanisms of action of these selfish elements. Using short- and long-read DNA and RNA sequencing, we identified the first fly "hot" donor locus of an endogenous retroviral element *rover*, highly active in the gut tissue. We then dissected the transcriptional landscape and local sequence and chromatin environment of all fixed *rover* copies present in the genome. This analysis offered insights into how locus-specific features allow active retrotransposon loci to escape repression, produce functional transcripts and mobilize in a somatic lineage.

Using this model system, my newly established team aims to further dissect the modes of retrotransposon regulation in the soma and the interplay between selfish genetic elements and tissue homeostasis *in vivo*.

*Speaker

Keywords: somatic transposition, Drosophila, LTR retrotransposons, endogenous retroviruses, ERVs, intestin, stem cells, aging

ASCT2, but not ASCT1, is a functional receptor for Syncytin-1-induced cell fusion

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Syncytin-1, a human protein of retroviral origin, is a remnant of an ancient infection. It has been exapted to fulfill a crucial role in human placenta morphogenesis. After the interaction with transmembrane protein ASCT2 (Alanine, Serine, Cysteine Transporter 2), Syncytin-1 triggers cell-to-cell fusion of trophoblasts into syncytiotrophoblast, the outermost layer of the human placenta. Dysregulation of Syncytin-1 has been implicated in various placental pathologies, including preeclampsia, low platelets syndrome, and intrauterine growth restriction.
Another transmembrane protein, ASCT1, has been proposed as an alternative cellular receptor for Syncytin-1. Together with the structurally similar ASCT2, both proteins have been reported as receptors for the RD114 and D-type retroviruses (RDR) interference group. However, the extent of their involvement in Syncytin-1-induced cell-to-cell fusion and their preference for RDR retroviruses require further investigation.
In this study, we examined the individual roles of ASCT1 and ASCT2 as receptors for Syncytin-1 using three quantitative assays. We compared the infection efficiency of Syncytin-1-pseudotyped virus on cells expressing either ASCT1 or ASCT2. Additionally, we evaluated the binding affinity of Syncytin-1 to ASCT2 and ASCT1 on the cell surface and, finally, assessed the fusogenic activity of Syncytin-1 following interaction with each receptor.
Our results indicate that ASCT1 exhibits at least two orders of magnitude lower receptor activity compared to ASCT2, casting doubt on its significance in syncytiotrophoblast differentiation. These findings underscore the pivotal role of ASCT2 in placental development and raise questions about the physiological relevance of ASCT1 in Syncytin-1-induced cell fusion.

Keywords: ASCT1, ASCT2, envelope, fusion, HERV, placenta, receptor, retrovirus

*Speaker

LINE1 expression and radio-response in rectal cancer cells

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All patients diagnosed with locally advanced rectal cancer in the UK routinely receive radiotherapy as a pre-operative neoadjuvant with varied response rates and _˜20% of tumours showing no response or disease progression. Effective markers to predict radiotherapy response would not only protect non-responsive patients from the debilitating effect of unnecessary treatment but also avoid the risks associated with surgery in fully responsive patients.
Ionising radiation drives a global decrease in methylation that is most pronounced at Long Interspersed Nuclear Elements-1 (LINE1s), a family of retrotransposons which are usually silenced in healthy cells. LINE1 hypomethylation is considered synonymous with LINE1 activation, however, LINE1 expression profiles have not been thoroughly characterised in irradiated cells. To investigate the relation between LINE1 expression and radio-response, we measured LINE1-5'UTR RNA in response to increasing radiation doses in two rectal cancer cell lines, the more sensitive SW837 and the more resistant HT55.

We observed an increase in LINE1-5'UTR RNA upon radiation in SW837 cells but not in HT55 cells. We then used CRISPR/Cas9 mediated HDR to generate SW837 cells stably expressing shRNAs designed on the endo453 sequence, a naturally occurring endogenous siRNA that specifically targets the LINE1 5'UTR. A decrease in LINE1-5'UTR containing RNAs and in ORF1P protein levels in SW837 cells constitutively expressing sh-endo453 are linked to an increased resistance to radiation compared to non-transfected cells.
We hypothesize that the rise in LINE1 expression contributes to increased radiosensitivity in SW837 cells. Further work is needed to validate these findings and determine whether LINE1/ORF1P expression can be utilised as a marker of response to radiation.

Keywords: Radioresponse, LINE1, endo453, rectal cancer, ORF1P

*Speaker

Dissecting the role of TE-mediated immune responses following epigenetic treatment in Acute Myeloid Leukemia (AML)

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The human genome has been continuously subjected to genome invasions of transposable elements (TEs) throughout evolution, which has led to almost two-thirds of our genome being TE-derived. Regulation of these elements, some of which are still mobile, is critical for genomic integrity and normal cellular function of gene expression programs, most of which are under strict epigenetic control. However, epigenetic dysregulations stemming from genome-wide loss of DNA methylation, global changes in histone modification marks and dysregulation of RNA modifications are all hallmarks of cancer and are typically synonymous with TE reactivation. Indeed, various studies demonstrate transcriptional activation of TEs in several cancer cell types. Our work aims to dissect the potential impacts of TEs on the host genome in a context which provides an epigenetically relaxed environment for their activation: acute myeloid leukaemia (AML). AML is a highly heterogeneous and aggressive haematological malignancy, characterised by relatively few genetic mutations compared to other cancers. Indeed, existing therapies are often accompanied by complications in drug toxicity, refraction and relapse, and so, there remains an unmet clinical need for the identification of new therapeutic targets and strategies for AML patients.

We aim to uncover how epigenetic therapies can be harnessed to help potentiate anti-tumour immune responses against endogenous TEs in AML. Specifically, we focus on DNA hypomethylating agents (5-AZA, Decitabine and DNMT1i) and those targeting histone modifications (HDACi and EZHi) to assess their combinatorial effects in promoting anti-tumour immune responses in patients of various genetic backgrounds. Our preliminary findings suggest that these agents result in differential TE and immune response activation in the presence and absence of DNMT3A mutations, the most commonly mutated epigenetic modifier in AML. Our next steps focus on uncovering the mechanism of 'viral mimicry' by interrogating the effects of TE-derived dsRNA and cDNAs on endogenous nucleic sensing pathways.

Keywords: Cancer, AML, TE, Epigenetics, Immunotherapy, Viral mimicry

*Speaker

Copy number alteration signal from plasma DNA LINE-1 targeted bisulfite sequencing: a new non-invasive multi-cancer detection marker

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Detection of circulating tumor DNA (ctDNA) allows non-invasive retrieval of tumor molecular profiles and disease monitoring. However, detecting small fractions of ctDNA shed when the tumor burden is reduced remains a challenge. It is therefore necessary to develop more sensitive biomarkers.

Our team has recently developed machine learning classifiers, based on cancer-associated LINE-1 hypomethylation, to discriminate with great accuracy cancer patients and healthy individuals from cell-free DNA (DIAMOND assay, Michel et al., medRxiv 2023). We observed that hypomethylation levels were similar in primary and metastatic tumor tissues, confirming that alteration of LINE-1 methylation is an early event in carcinogenesis. However, we detected higher hypomethylation levels in metastatic compared to localized stages in plasma, reflecting the fraction of circulating tumor DNA, which correlates with the tumor burden. It has also been shown that copy number alteration (CNA) detected from cell-free DNA strongly correlates with tumor burden and can be inferred from PCR-based targeted LINE-1 sequencing.

I investigated whether CNA signal could be extracted from the DIAMOND data and used as a multicancer biomarker.I developed a pipeline quantifying CNA on a genome-wide scale (Z score). This approach was validated on cell lines that were also characterized with CGH arrays, a classical method for CNA analysis. In plasma samples, high Z scores were observed specifically in cancer samples. Z score and hypomethylation levels were only moderately statistically correlated, demonstrating that these are partially independent markers providing distinct signals. Therefore, I created a 2-step classifier using the DNA methylation model and the CNA-scores, which improved cancer detection, particularly for localized breast cancer.

*Speaker

Altogether, our results support the idea that LINE-1-based CNA, along with hypomethylation, can be used as a non-invasive multi-cancer biomarker.

Keywords: L1 elements, Copy number alteration, ctDNA, Multi, cancer, biomarker, Liquid biopsy, Machine learning

TE control and epigenetics

Role of retrotransposon RNA-binding proteins in Ty1 activity: proteomic identification by ChIRP-MS and comprehensive characterization

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LTR retrotransposons are generally repressed in mammals and do not lead to new genome insertions. Their transcription reactivation is a feature of malignant cells and aging, and is associated with genome instability. Retrotransposon RNA is a key intermediate in the replication of LTR retrotransposons as it serves as template for both translation and reverse-transcription. Yet, we know little about the proteome involved in RNA retrotransposon fate. Moreover, while genomic instability is in part due to retrotransposition, it remains unclear whether the presence of the transcript itself also participates, for instance by inducing replication stress or nucleic acid secondary structures. Therefore, proteins that bind LTR retrotransposon RNA could be pivotal in genome instability linked to retrotransposon activity. Using the LTR retrotransposon model Ty1 in *S. cerevisiae*, we have set up a new method named Comprehensive Identification of RNA-binding Proteins by Mass Spectrometry (ChIRP-MS) to identify such Ty1 RNA-binding proteins. Based on stringent criteria, we selected a total of 29 candidates associated with Ty1 RNA, 66% of which had never before been identified as regulators of retrotransposition. We have begun to study the impact of Ty1 RNA-binding proteins on Ty1 activity by constructing deletion mutants of selected candidates. We will present a first analysis of these mutants on the different steps of the Ty1 cycle. Our study is promising in deciphering the function of these candidates on Ty1 activity and genome instability.

Keywords: LTR retrotransposon, Ty1, RNA, binding proteins, yeast, proteomics, functional analysis

*Speaker

Deciphering the role(s) of PRC2-Ezl1 mediated histone H3 modifications in programmed DNA elimination in Paramecium

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In most eukaryotes, the genetic information remains constant throughout their life cycle. Yet this is not a universal rule. In the unicellular eukaryote *Paramecium,* massive and reproducible elimination of transposable elements and their single-copy remnants occurs, at each sexual cycle, during the development of somatic macronucleus from the germline micronucleus. Understanding how such diverse sequences are recognized and eliminated remains challenging. The DNA elimination process involves small RNA-guided deposition of H3K9me3 and H3K27me3 catalyzed by PRC2-Ezl1 onto transposable elements. In this project, we aim at understanding the precise roles of these two repressive histone modifications. Here, we characterize Chromo2, a chromodomain-containing protein, that we showed is required *in vivo* for correct H3K9me3 and H3K27me3 accumulation and DNA elimination. Using a functional tagged protein, we found that Chromo2 exclusively localizes in the developing macronucleus and that it associates with transposable elements through ChIP-qPCR experiments. Through pulldown and quantitative mass spectrometry, we identified two Chromo2-interacting proteins, whose silencing phenocopy *CHROMO2* knockdown, suggesting a joint action. Further work will shed light on the precise role of Chromo2 and its partners, in relation with histone modifications, during the process of DNA elimination in *Paramecium.*

Keywords: Epigenetics, Histones, Genome stability

*Speaker

Limited impact of the siRNA pathway on transposable element expression in Aedes aegypti

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Transposable elements (TEs) are DNA sequences that can change their position within a genome. In the germline of arthropods, post-transcriptional regulation of TE expression is mainly mediated by the Piwi-interacting RNA (piRNA) pathway. piRNAs are small RNAs of 24-30 nucleotides (nt) in length produced from genomic precursor transcripts as well as through a 'ping-pong' amplification cycle. In somatic tissues, certain insects, such as *Drosophila*, instead rely on the small interfering RNA (siRNA) pathway as a key regulator of TE expression. siRNAs are 21-nt small RNAs produced from double-stranded RNA by the endonuclease Dicer2, which guides an RNA-induced silencing complex to degrade a target RNA. However, whether the siRNA pathway also regulates TE expression in the mosquito *Aedes aegypti*, a medically important vector species with abundant somatic piRNAs, is unknown.

To address this question, we investigated the expression of TEs and small RNAs in both somatic and gonadal tissues of a *Dicer2* mutant line of *Ae. aegypti* and its wild-type counterpart. Our results show a modified pattern of TE expression and a decrease in TE-derived 21-nt small RNAs in the *Dicer2* mutant line. Despite that, no major shift of TE transcript abundance is observed, nor any compensation by increased piRNA pathway activity. Interestingly, the lack of a functional siRNA pathway also causes perturbations in ping-pong activity and the expression of certain piRNA-associated genes.

In conclusion, the mosquito *Ae. aegypti* produces siRNAs targeting TEs but these lack a critical role in the regulation of TE expression both in somatic and in gonadal tissues. Whether the lack of Dicer2 affects TE activity remains to be studied.

Keywords: yellow fever, mosquito, small RNA, RNAseq, pingpong cycle, piRNA, Dicer2

*Speaker

HEBE Project: Healthy aging versus inflamm-aging: the role of physical exercise in modulating LINE-1 methylation as a potential biomarker for lifestyle improvement

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Inflammaging refers to the chronic low-grade inflammation that occurs with aging and cellular senescence, and it is linked to various diseases. Understanding the markers involved in chronic inflammation and aging, as well as their interaction with environmental factors and inner control mechanisms, can provide crucial tools for assessing the resilience (i.e. the ability to adapt and improve) of the human body, particularly in the presence of degenerative conditions or vulnerable life stages, that place the individual and the community to which she/he belongs in a state of potential fragility.
In this context, HEBE (*Healthy aging versus inflammaging: the role of physical Exercise in modulating the Biomarkers of age-associated and Environmentally determined chronic diseases*) focuses on exercise, along with nutritional and lifestyles recommendations, to reduce systemic inflammation and promote healthy aging.

Healthy lifestyle recommendations were provided to University of Milan employees, and changes in quality of life and well-being were assessed using questionnaires. The first 100 eligible subjects, who expressed their willingness to participate, underwent a trial of a personalized exercise protocol based on clinical and objective assessments (ClinicalTrials.gov ID: NCT05815732). Blood samples were collected at baseline (T0) and follow-up (T1) to evaluate the effect of exercise on LINE-1 methylation according to individual characteristics such as anthropometric, cardiovascular, and metabolic health.

LINE-1 methylation was increased in T1 versus T0. Moreover, body mass index (BMI) modified the effect of lifestyle improvement on LINE-1 methylation levels. In particular, an increase in *LINE-1* methylation was observed in subjects with BMI < 25.
The results of this project will provide information on the link between *LINE-1* methylation, physical activity, and inflammaging. The multidisciplinary approach of Project HEBE offers a

*Speaker

platform for further research on the relationship between repetitive element methylation and health maintenance.

Keywords: LINE, 1 methylation, physical exercise, Inflammaging

A new approach to assess the relationship between the exposome, LINE-1 methylation, and health

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Exposure factors may trigger epigenetic changes, notably in DNA methylation of repetitive elements (REs). For instance, LINE-1 elements typically methylated in normal cells, can become active in response to environmental cues. Prior research has predominantly assumed that all epigenetic changes are detrimental, but we suggest a novel perspective: some environmentally-induced changes could be physiological responses facilitating individual adaptation.
In 185 subjects with a Body Mass Index > 25, we evaluated through regression models the association between 1-week exposure to particulate matter ≤ 10 μm (PM10) and LINE-1 methylation (assessed by Pyrosequencing). We used the estimated regression function to predict values for each study subject and we calculated the relative percent difference (PD) between the measured (i.e., observed) LINE-1 methylation and the predicted (i.e., expected) from the model. We hypothesize that the greater the difference between the observed and the expected, the greater the inability of the subject to adapt to external stimuli.

We observed a negative association between PM10 and LINE-1 methylation (β = -0.038; 95% CI -0.052; -0.0233; p-value < 0.0001), in agreement with previous evidence. Applying the new approach that we are proposing, we calculated the PD between the measured LINE-1 methylation values and the estimates predicted from the model and we identified subjects showing a negative PD (i.e., observed values lower than estimated ones) higher than 5%. The health status of these subjects was associated with a 4-fold greater risk of metabolic syndrome when compared to all other subjects (Odds Ratio (OR) = 3.78; 95% CI 1.28-11.20; p-value 0.016) and a 9-fold higher risk of hypertension (OR = 8.97; 95% CI 1.15-69.82; p-value 0.036).
The proposed approach will be extended (for example considering a wide range of environmental exposures and lifestyle factors) and thoroughly tested in a population of more than 6,000 subjects in the MAMELI project ("MApping the Methylation of repetitive elements to track the Exposome effects on health: the city of Legnano as a LIving lab") which is about to start and has been funded by the European Research Council (project code: ERC-2022-COG-101086988). If confirmed, our hypothesis will pave the way to support the notion that REs are fundamental components for monitoring individual adaptability to environmental stimuli.

*Speaker

Keywords: Epigenetics, DNA methylation, Exposome, Adaptation, Misadaptation

Transposon control in satellite cells during quiescence and differentiation

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Satellite cells are unipotent precursors to skeletal muscle cells and are responsible for the ability of the muscle to regenerate. These dormant cells play a vital role in the maintenance and the regeneration of the skeletal muscles and are an important target of therapies. Our RNA-seq data shows that transposable element (TE) activity changes significantly between the states of quiescence and early activation of the satellite cells. These changes are accompanied by a marked downregulation of the Krüppel-associated box zinc finger protein (KRAB-ZFP) gene clusters, a family of transcriptional repressors that have evolved in vertebrates to repress evolving TEs. Many of these ZFPs share a main corepressor, KAP1, which brings about genetic silencing of the TEs via histone methylation. We have identified several targets of ZFPs that may play a role in the quiescent state of the satellite cells. We are particularly interested in understanding the KAP1-ZFP-TE interaction in the satellite cells, the resulting biological changes that take place and how this influences the quiescent/activation states. We show that KAP1 knock-down in isolated myofibers increases the expression of Pax7, a quiescent marker of the satellite cells. We conclude that the activity of KAP1 and specific ZFPs may have important roles in the quiescent state of the satellite cells as well as their capacity to differentiate.

Keywords: KZFP, transposable element, satellite cell, skeletal muscle

*Speaker

Novel SOX transcription factors as L1 modulators in neurons

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Long interspersed element-1 (L1) is the only autonomously mobile human retrotransposon. The L1 5 untranslated region (5UTR) contains an internal sense promoter that drives the expression of a bicistronic mRNA encoding the L1 retrotransposition machinery proteins ORF1p and ORF2p. The L1 5UTR also possesses two SOX binding sites. Previous studies have described SOX proteins as L1 modulators. To date, L1 transcriptional activity has been identified in various somatic tissues, with the brain being an apparent hotspot for L1 mRNA expression and retrotransposition. However, the mechanisms by which SOX factors modulate L1 in the brain remain unclear.

To address this question, we generated novel L1-EGFP retrotransposition reporter and L1 5UTR-EGFP promoter reporter assays. We found that SOX6 overexpression significantly increases endogenous L1 expression and ORF1p abundance, as well as L1 reporter mobility and transcriptional activity in HeLa. Mutations in the first SOX binding site of the L1 5UTR significantly reduced these effects. When deleting the DNA binding domain of the SOX6 protein, we observe a reduction in L1 promoter reporter transcriptional activation. Lastly, we translated these findings into a recently established model of human induced pluripotent stem cell (hiPSCs)-derived neurons (i3Neurons). We demonstrate that i3Neurons express high endogenous L1 mRNA and ORF1p. Overexpression of SOX6 in fully mature and functional i3Neurons enhances ORF1p expression and L1 5UTR reporter activity, compared to a SOX6 DNA binding domain mutant. In addition, we confirmed in our neuronal model previous reports of SOX2-mediated L1 transcriptional repression.

These findings together provide a deeper insight into SOX-mediated L1 regulation, presenting SOX6 as a novel L1 transcriptional modulator in human neurons.

Keywords: L1 modulation, SOX factors, Neurons, human iPSC

*Speaker

CDCA7: A Key Contributor to Transposon DNA Methylation in Natural Arabidopsis Populations

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Understanding the genetic mechanisms that control transposon activity is key to unraveling sources of genetic variation. In this study, we looked for genetic determinants of CG methylation, an epigenetic mark suppressing transposon expression. Our genome-wide association study in Arabidopsis identified the *CELL DIVISION CYCLE ASSOCIATED 7* (*CDCA7*) gene as a crucial regulator of CG methylation at transposons. We show that CDCA7 binds to DECREASED DNA METHYLATION 1 (DDM1), known to repress transposon activity. Interestingly, in vertebrates, the DDM1 ortholog LSH/HELLS interacts with CDCA7, although the interdependence of their functions is not fully understood. Our analysis of *cdca7* and *ddm1* null mutants in plants reveals that DDM1 activity is largely dependent on CDCA7. Genetic variation in the *CDCA7* promoter region appears to fine-tune this pathway in natural populations. We discovered two divergent *CDCA7* alleles, arising from an ancestral haplotype, that confer opposite phenotypic outcomes and have become prevalent in distinct environmental settings. In sum, our work shows that CDCA7 acts as a controller of global DNA methylation levels in natural populations, with the potential to modulate numerous epigenetically controlled traits, including transposon activity.

Keywords: DNA methylation, genetic variation, epigenetic regulation, transposon silencing, GWAS, CDCA7, DDM1

*Speaker

Determining the genetic, environmental and developmental basis of heritable transposition in Arabidopsis thaliana

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Transposable elements (TEs) are ubiquitous DNA sequences capable of self-propagating across genomes. To limit their mutagenic potential, TEs are typically controlled by epigenetic mechanisms, including DNA methylation in plants and mammals. Nonetheless, TEs eventually evade their epigenetic silencing and transpose. Using Arabidopsis thaliana, we recently reported that the mobilome of this species spans more than 150 TE families, and that the transposition rate in nature varies across ecotypes in association with genetic and environmental factors. Nonetheless, only a few Arabidopsis TEs have been observed to transpose experimentally so far, raising the question of what genetic and environmental factors control transposition. Here, we set out to measure the rate of heritable transposition using a comprehensive panel of genetically diverse Arabidopsis plants subjected to a range of environmental conditions. Overall, we detected significant heritable mobilization for at least 34 TE families, spanning both retrotransposons and DNA transposons. Importantly, we show that epigenetic perturbations are sufficient to trigger transposition bursts in a TE-specific manner, with the environment playing a secondary, modulatory role in TE mobilization. Unexpectedly, we found that some epigenetic mutants triggering extensive DNA hypomethylation, such as those affecting the chromomethylases CMT2 and CMT3, are dispensable for controlling transposition. To investigate the molecular mechanism underlying TE activity, we studied the developmental window where heritable transcription takes place, by collecting seeds derived from flowers from the same or different inflorescence, and from different stems. Overall, the segregation pattern of new insertions reveals that distinct TEs transpose at different reproductive stages, and that the environment shapes the developmental window in which transposition is occurring. Our work provides a first genetic and environmental (GxE) interaction map of transposition and sets the basis for uncovering the molecular mechanisms controlling the developmental specificity of TE reactivation.

Keywords: Arabidopsis thaliana, heritable transposition, DNA methylation, environmental cues, reproductive tissue

*Speaker

Genome-wide mapping of LINE-1 elements and their methylation states through targeted long-read sequencing

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The human genome is mosaic in tissues and shaped by a myriad of mutational processes. Our group studies its only active autonomous retrotransposon LINE-1(L1), which generates novel insertions through a "copy and paste" mechanism of RNA reverse transcription. These *de novo* insertions can interrupt genes or alter their expression, generate chromosomal rearrangements, or generally contribute to DNA damage and genome instability. Somatic L1 activity is best studied in cancers, with the L1 protein ORF1p present across a variety of cancer types, while recent work has demonstrated somatic activity in normal tissue including the brain and the colon. 17% of the human genome is made up of L1 sequence, yet only about 100 copies are intact and capable of retrotransposition in an individual. Many active L1 loci ("hot" L1) are genetic variants in human populations and thus differ from individual to individual. In a given tissue or cell type, a subset of these may be expressed. Defining active L1 loci has been challenging due in-part to the limited abilities of short read sequencing to accurately map to and cover the full length of L1 insertions, and the difficulties in then combining that sequence data with locus specific methylation data. Here we present a Cas9 targeted long read approach using Nanopore which allows us to sequence the entirety of genomic L1 insertions with their flanking regions and capture the methylation status of each locus directly from the genomic DNA. We combine this protocol with a novel *in silico* pipeline to analyze targeted Nanopore reads from repeat elements and can integrate it with existing L1 insertion detection tools. This technique will enable the field to continue to refine our understanding of "hot" L1 activity.

Keywords: LINE, 1, Sequencing, Nanopore, Methylation, Long, Read

*Speaker

Stem cells protection from and by transposable elements

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The maintenance of mammalian tissue architecture relies on the constant renewal of differentiated cells by division of stem cells. Thus, if most differentiated cells can be considered expendable, because replaceable, stem cells need to be specifically shielded from insults. For example, stem cells are under pressure to fine-tune the expression of transposable elements (TEs). Published work, as well as our preliminary data, indicate that RNA interference (RNAi) mediated by a specialized Dicer protein or its antiviral isoform aviD, transcriptionally regulates certain TE subfamilies in stem cells. This project aims at **deciphering the role of RNAi in controlling transposable elements in stem cells**.
To tackle this question, we first aim to unravel the molecular mechanism(s) of TE control in stem cells. We generated mouse embryonic stem cells (mESC) lines encoding for degron-tagged versions of Dicer or aviD, allowing auxin-inducible loss of RNAi. Our preliminary data suggest that certain TE subfamilies are de-repressed upon depletion of Dicer and aviD. We now aim to determine the identity of RNAi-controlled TEs, as well as understand the molecular mechanism of control. Published work indicate that certain protein-coding TEs can hinder viral infection, suggesting that TE expression can, in specific contexts, be beneficial for stem cells. We are exploring this question in the context of neural stem cells infected with herpes simplex virus 1 (HSV-1). Our preliminary data suggest that evolutionary young TEs documented to counteract HSV infection may be upregulated during infection in a RNAi-dependent manner.
Overall, we aim at unraveling the means of RNAi-driven TE control in stem cells and understanding the putative role of such mechanism during viral infection.

Keywords: Stem cells, transposable elements, RNA interference, innate antiviral immunity

*Speaker

Oxidative stress response heterogeneity, at single cell and lineage level, could influence activation of Integrative and Conjugative Element ICEclc

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Bacteria adaptability is closely associated with horizontal gene transfer (HGT), often facilitated by mobile genetic elements that contribute to the plasticity of bacterial genomes. While the mechanisms underlying HGT are well-documented, the corresponding regulatory systems and the impact of environmental signals on gene transfer are less understood.
Here, we focus on the Integrative and Conjugative Element ICE*clc*. We investigate the environmental signals influencing its activation. ICE*clc* carries genes responsible for the degradation of pollutants, notably the aromatic compound 3-chlorobenzoate (3CBA). Its activation is limited to a small subset of 'transfer competent' (tc) cells. Interestingly, the activation rate peaks in stationary phase for cells grown with 3CBA (2-5% of cells), and appears to be specific, as few tc cells are detected with the related compound benzoate. Growth with 5 mM 3CBA also induces a high level of intracellular oxidative stress, which is not observed with benzoate.
Our primary hypothesis is that the high ROS levels detected in 3CBA-grown cells influence tc cell formation, either directly through the activation of ICEclc regulators or indirectly through other cellular factors such as RpoS. Specifically, we propose that cells experiencing higher-than-average oxidative stress are more likely to initiate the tc program. To test this hypothesis, we used double-reporter strains, with one fluorescent gene fused to an ICE*clc* promoter to serve as a proxy for its activation, and the other fused to one of six selected oxidative stress response promoters. We quantified both fluorescence signals at the single-cell level using flow cytometry as a function of growth substrate, and employed time-lapse microscopy to assess a potential temporal link between oxidative stress and tc appearance. Our results suggest that tc cells preferentially appear in cells with a higher oxidative stress response, which could be linked to a higher oxidative stress response experienced by their lineage ancestors.

Keywords: ICE, Regulation, Oxidative Stress, Timelapse microscopy, Flowcytometry

*Speaker

How Does Wolffia (Lemnaceae), the Smallest Flowering Plant, Silence Transposable Elements?

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Transposable elements (TEs) or transposons, are mobile DNA sequences capable of replicate within host genomes. TEs are silenced by numerous epigenetic mechanisms, being the deposition of DNA methylation one of the most relevant in plants. In angiosperms, the RNA-directed DNA methylation (RdDM) pathway plays an important role in depositing de novo DNA methylation on TEs through the production of 24nt-siRNAs. However, the duckweed family (Lemnaceae) appears to be an exception. Duckweeds represent the smallest and fastest growing flowering plants known to date, thanks to rapid asexual clonal propagation. During clonal propagation, several of its members show no expression of RdDM components, low levels of 24nt-siRNAs, and low DNA methylation associated with RdDM activity although TEs remain silenced. To fully understand TE silencing mechanisms in duckweeds, we have expanded our investigation to one of the most recent lineages of the family: *Wolffia brasiliensis*. *Wolffia brasiliensis* has a relatively high TE content and show recent bursts of transposition compared to the other duckweeds investigated. Like other duckweeds, *Wolffia* exhibits no RdDM expression and associated DNA methylation signatures. However, in contrast to the other species, 22nt-siRNAs can be found arising from TE loci alongside 24nt-siRNA in several instances. In plants, 22nt siRNAs are associated with Post-Transcriptional Gene Silencing (PTGS) while 24nt are involved in RdDM mediated Transcriptional Gene Silencing (TGS), two pathways considered as mutually exclusive. Whether both siRNA classes share a common source of siRNA precursors, or the different silencing pathways in *Wolffia* follow a tissue specific expression pattern, remains unknown so far. These unique features of duckweeds, and *Wolffia* in particular, offer an exceptional opportunity to investigate TE silencing and their interplay with their hosts in non-model plant organisms.

Keywords: TEAnnotation, NonModel, Silencing, Epigenetics

*Speaker

Exploring the impact of transposable element activity on genome stability and organization during meiosis

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Context-specific TE control is crucial to preserve the integrity of the host genome. Repression of TEs is particularly important during gametogenesis, as mutations would be transmitted to the next generation and would compromise the fitness of the progeny. The fetal stages of gametogenesis are characterized by an extensive erasure of DNA methylation, leading to a transient relaxation of the chromatin that could result in derepression and mobilization of TEs. To avoid such threat, DNA methylation is rapidly reestablished by the action of specialized *de novo* DNA methyltransferases and maintained after birth during spermatogenesis and in the mature spermatozoa. Meiosis is an important process during spermatogenesis, in which haploid cells are generated and homologous recombination causes genetic exchange between homologs. Chromatin organization is highly dynamic during early meiosis and plays a critical role in meiotic progression. It has been previously shown that failure to methylate young TEs during fetal gametogenesis leads to reactivation of young TEs. It is not immediately detrimental but causes damage after birth: meiotic chromosome pairing is altered, leading to apoptosis and male sterility. We aim at deciphering the mechanisms driving this developmental arrest, using two mouse models of TE reactivation: a) Deficient DNA methylation using DNMT3C-KO; or b) Temporally controlled CRISPR-based activation. We will 1) investigate the impact of TE activity on meiotic chromatin landscape and distribution of recombination sites, and meiotic chromosome conformation and 2) control TE reactivation in space and time to assess the impact of different TE subfamilies. Based on previous findings, we hypothesize that meiotic arrest is not due to retrotransposition, but hypomethylated young TEs acquire a relaxed chromatin environment that attracts the meiotic HR machinery, leading to synaptic failure and non-homologous recombination. Additionally, TE reactivation could impact neighboring genes, leading to aberrant transcriptional activation at a stage otherwise characterized by transcriptional shutdown.

Keywords: TEs, DNA methylation, meiosis, homologous recombination, synapsis, developmental arrest, chromatin, transcription, CRISPRa

*Speaker

Why using the right genome assembly matters.

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The reference genome GRCm39 is commonly used for alignment of genomic data in studies of mouse genetics. However, this assembly was generated using C57BL/6J mice, and many in vitro studies use E14 mouse embryonic stem cells derived from 129/Ola mice. A recent study identified over 30,000 genomic insertions and 27,000 deletions in 129 mice compared to the GRCm39 reference (Ferraj *et al.*, 2023). Furthermore, even within sub-strains of 129 mice there is further genetic diversity. Transposable elements are the main source of insertions, and their high sequence homology can promote other types of structural variation, such as deletions or translocations. Hence, a high-quality strain-specific reference genome is needed to obtain an accurate reflection of the abundance and distribution of repetitive elements and enable study of their functional roles. Using nanopore direct whole genome DNA sequencing and the 129/SvImJ reference generated by Ferraj et al (2023), we have generated a novel assembly of the E14 genome. Here, we identify a further 3000 novel insertions over 100bp compared to 129S1/SvImJ reference. This suggests that the GRCm38/39 references are an inaccurate reflection of the number, type, and location of TE loci in the E14 genome, and may result in incorrect assignment of TE-derived reads in genomic datasets.

Keywords: epigenetic regulation, structural variation, genome assembly

*Speaker

Unravelling Epigenetic Dynamics in Spirodela polyrhiza: Insights into Transposable Element Regulation

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In flowering plants, the regulation of transposable elements (TEs) involves various molecular processes that lead to the formation of heterochromatin. This state is characterized by heightened DNA methylation (mDNA) and specific histone modifications (e.g. H3K9me2). To ensure TE specificity, 24nt small interfering RNAs (siRNAs) guide mDNA deposition through the RNA-directed DNA methylation (RdDM) pathway. Once established, mDNA can persist independently of siRNAs due to a positive feedback loop with H3K9me2. In contrast to the widespread occurrence of RdDM in the vegetative and sexual tissues of flowering plants, Spirodela polyrhiza, a member of the Lemnaceae family, exhibits a distinct pattern. This aquatic specie displays reduced mDNA, minimal RdDM expression, and a near absence of 24-nt siRNAs during clonal vegetative reproduction. Furthermore, some key components of RdDM, mDNA maintenance and RNA silencing are conspicuously absent from its genome. The characterization of the TE epigenetic landscape in Spirodela reveals a unique pattern where the loss of mDNA and H3K9me2 coincides with TE decay in gene-rich regions. However, remnants of TEs persist in a silenced state, marked by H3K9me1. In contrast, the few intact TEs exhibit significant DNA methylation, H3K9me2, and, interestingly, 21- and 22-nt siRNAs, resembling the patterns observed TEs subjected to RdDM in other angiosperms. Notably, Spirodela produces 22-nt siRNAs from transiently expressed double-stranded RNA, even in the absence of DCL2, an siRNA production-associated enzyme. While unlikely involved in RdDM, this suggests an alternative silencing pathway in Spirodela, possibly subject to tissue or developmental regulation. These findings underscore the importance of diverse plant models in elucidating silencing pathway complexities, enhancing our understanding of plant epigenetics.

Keywords: transposon, RdDM, mDNA, chromatin, Spirodela

*Speaker

Investigating the role of ZNF93 in L1 promoter DNA methylation

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LINE-1 (L1) retrotransposons are the only active and autonomous transposable element in the human genome. We recently analyzed the DNA methylation levels of their promoter region at individual loci, in a varied panel of human cell lines. We found that embryonic cells exhibit a unique profile with the youngest primate-specific L1 families being hypomethylated but older ones being hypermethylated. Notably, the transition seems to occur within the L1PA3 family, which possesses two versions of the promoter. In an older subgroup, the L1 promoter contains a binding site for ZNF93, a Krüppel-associated box (KRAB) domain-containing zinc-finger protein, which can recruit Kap1/TRIM28, leading to the formation of repressive chromatin. By contrast, a younger L1PA3 subgroup shows a deletion encompassing the ZNF93 binding site. We confirmed that these two L1PA3 subgroups recapitulate the differential methylation observed between young vs old primate-specific L1 elements in embryonic cells, the loss of methylation being associated with the deletion of the ZNF93 binding site. To test whether ZNF93 plays a direct role in L1 promoter methylation, we generated *ZNF93* knock-out clones and assessed DNA methylation of each individual primate-specific L1 promoter using our recently developed strategy, bs-ATLAS-seq. We did not observe significant alterations of L1 DNA methylation profiles, indicating that ZNF93 is not a necessary factor to maintain DNA methylation of L1 promoters from L1PA3 and older primate-specific families in these cells. However, it is still possible that ZNF93 is involved in the establishment of their methylation profiles. AJD, CP and SL contributed equally. This work was supported by Agence Nationale de la Recherche (ANR-11-LABX-0028; ANR-11-LABX-0071; ANR-15-IDEX-0001; ANR-16-CE12-0020; ANR-18-IDEX-0001; ANR-19-CE12-0032; ANR-21-CE12-0001), Fondation pour la Recherche Médicale (DEQ20180339170), Institut National Du Cancer (INCa PLBIO 2020-095), Fondation ARC (PGA1/RF20180206807), grants from the Canceropôle PACA, INCa, Region Sud (Projet Emergence), INSERM (GOLD Cross-cutting Program on Genomic Variability), CNRS (GDR 3546).

Keywords: LINE, 1, retrotransposon, epigenetic control, promoter methylation, ZNF93

*Speaker

Chromatin self-defence against Transposable Elements: role of a specific histone variant during D. melanogaster oogenesis

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Transposable Elements (TEs) represent 15% of *D. melanogaster* genome and many families are still active. TEs expression and insertion is particularly deleterious in the germline since mutations are transmitted to the progeny. Most often TEs over-expression induces sterility in fruit flies. Fortunately, the genome has its own immune system based on small non-coding RNAs targeting TEs by sequence complementarity: piRNAs and siRNAs. Associated with Piwi family proteins, they inhibit TEs expression at the transcriptional or post-transcriptional level. Although the role of epigenetic marks such as H3K9me3 in TEs silencing and piRNAs expression has been extensively documented, **little is known about the use of histone variants to control TEs expression**.
Histone variants strongly impact nucleosome accessibility, playing important functions in eukaryotic genome regulation: transcription control, DNA repair, cell division, ... Interestingly, while mammals have up to 10 H2A variants with different functions, *D. melanogaster* has only one, called H2Av. It is known to regulate transcription, to deposit heterochromatin and to mark sites of DNA double-strand breaks when phosphorylated.

In the lab, we have uncovered a role for H2Av in oogenesis, since its depletion leads to a developmental delay, oogenesis arrest and sterility. Moreover, TEs (such as *I-element*, *Max-element* and *gypsy12*) are highly expressed in H2Av mutants and the nuage, known as the "piRNA factory", does not assemble. Finally, we show that H2Av germline mutants accumulate replication stress and activate DNA damage checkpoints upstream of p53, leading to oogenesis arrest.
Altogether, we have evidences of a new role of H2Av in the defense and maintenance of *D. melanogaster* genome integrity in the germline, by controlling TEs expression.

Keywords: Histone variants, Chromatin, DNA damage, Oogenesis, Transposons

*Speaker

Crosstalk between the innate immune system and retroelements in human induced pluripotent stem cells.

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Transposable Elements (TEs) constitute both a potential threat to genome integrity and a ready source of genetic material for co-option. In development, TEs are subject to epigenetic silencing through TRIM28, KRAB-zinc finger proteins and the Human Silencing Hub (HUSH) complex. We recently showed that the HUSH complex is an epigenetic regulator of Long INterspersed Element-1s (LINE1s) and long terminal repeats (LTRs) and regulates the type I interferon (IFN) response in adult tissues. Inactivation of this complex leads to the cytoplasmic accumulation of LINE1 RNAs; a double-stranded RNA (dsRNA)-sensing dependent IFN response; and induction of IFN-stimulated genes (ISGs).

Human induced pluripotent stem cells (iPSCs) are canonically insensitive to IFNs. Instead showing intrinsic expression of a subset of ISGs and anti-viral RNAi. These cells show increased expression of LINE1 mRNA. Based on this, we hypothesise that components of the type I IFN induction pathway are epigenetically repressed in pluripotent stem cells to protect from fatal interactions caused by retroelement-mediated induction of type I IFN. We use an isogenic IPSC and NPC comparison model to identify changes in IFN regulation between IFN sensitive and insensitive cells.

Our data suggest that human iPSCs are mildly responsive to the dsRNA analogue, Poly(I:C), with upregulation of some ISGs. Depletion of MPP8, a component of the HUSH complex, also leads to partial ISG upregulation, which appears to act independently of both the type I IFN receptor (IFNAR) and of downstream JAK STAT signalling. By integrating RNA sequencing and chromatin profiling data, we are assessing how type I IFN is regulated in early development. This work aims to illuminate how cells may transition to a state of immune evasion.

Keywords: HUSH Complex, Interferon, LINE1, LTR, Stem Cells, IPSC, Innate Immunity

*Speaker

Transposable Elements silencing by the histone demethylase dLSD1 is required to achieve normal wing size

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How organs achieve their appropriate size represents a fundamental question in biology.Chromatin plays an important role in regulating gene expression during development, including the determination of organ size. In *Drosophila melanogaster*, the wings provide an ideal model system to explore the molecular mechanisms underlying organ growth.

Here, we demonstrate that deficiency of a key chromatin regulator, the histone demethylase *dLsd1* leads to reduced wing size due to a decreased cell number. Our work shows that dLsd1 depletion causes cell cycle arrest and apoptosis associated to an increased DNA damage. Through genomic analysis, we show that these phenotypes correlate with increased transposon expression and mobilization. Conversely, administering a reverse transcriptase inhibitor to dLsd1 null mutants results in partial restoration of normal wing size concomitant with reduced transposon mobilization.
In summary, our work shows the critical role of dLsd1 in modulating Drosophila wing size through the suppression of transposon expression and mobility.

Keywords: Transposable elements, silencing, chromatin, LSD1, drosophila

*Speaker

Identifying regulators of LINE-1 expression using FACS-based CRISPR Screens

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Approximately one third of the human genome is composed of repetitive DNA elements derived from retrotransposons. Long interspersed element-1 (LINE-1) is the only active, protein-coding retrotransposon in humans, and its retrotransposition is normally repressed in somatic cells via several mechanisms, including epigenetic silencing. In contrast, LINE-1 overexpression and *de-novo* LINE-1 insertions are commonly detected in human cancers with *TP53* mutations. A retrotransposition competent copy of LINE-1 encodes an RNA binding protein (ORF1p), which is produced abundantly, and a protein with endonuclease (EN) and reverse transcriptase (RT) activities (ORF2p). Hence, the detection of ORF1p is ideal for detecting expression of retrotransposition competent LINE-1 copies in human cells. To identify suppressors of LINE-1 expression, we have performed genome-wide forward genetic CRIPSR screens using intracellular staining of endogenous ORF1p to detect LINE-1-expressing cells in knockout cell pools using fluorescence-activated cell sorting (FACS). Our screen revealed previously identified epigenetics suppressors of LINE-1 expression, including DNA methyltransferases and factors in the HUSH complex, as well as additional mechanisms of LINE-1 suppression. Together, these studies will thereby identify factors that regulate LINE-1, providing new insights into the regulation mechanisms of LINE-1 expression and its impact on human cancers.

Keywords: LINE1, Epigenetics, CRISPR Screens

*Speaker

Bruno is a host co-factor that establishes natural variation in P-element transposition

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Transposable elements recurrently invade new host genomes through horizontal transfer, often between distantly related species. These horizontal transfer events are characterized by a burst of transposition in the naive host genome, followed by the evolution of piRNA mediated silencing that brings the invader under host control. However, lost in this story of evolving host defense is the initial requirement for positive regulation of the TE by the host genome, in the form of host-encoded factors that promote the transcription, splicing and translation of TE derived mRNA. Variation in these host co-factors both within and between species is predicted to be highly influential of TE invasions following horizontal transfer, yet this variation remains almost entirely uncharacterized.
We recently discovered that Bruno likely represents a host-cofactor that promotes the transposition of P-elements in *Drosophila melanogaster*. We uncovered *bruno* through QTL mapping of natural variation in hybrid dysgenesis: a sterility syndrome resulting from *P*-element transposition. *bruno* encodes an RNA binding protein expressed in the female germline, with known functions in regulation of mRNA translation and alternative splicing. Consistent with a model in which Bruno regulates *P*-element mRNA, Bruno binding sites occur within *P*-element transcripts, and P-element mRNA abundance and splicing are altered in *bruno* heterozygotes. Furthermore, reduced bruno dosage suppresses both *P*-element excision and dysgenic sterility. Our work represents the first demonstration of natural variation in a host-cofactor of transposition. It further reveals how such variation can have a major effect on host fitness when new TEs invade: with permissive genotypes suffering increased transposition rates and reduced fitness.

Keywords: P, element, hybrid dysgenesis, RNA binding proteins, alternative splicing

*Speaker

Small RNA biosynthesis in Duckweeds

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In flowering plants, small RNA pathways are the key components of TE silencing and control. At the transcription level, TEs are silenced by a mechanism called RNA-directed DNA methylation (RdDM), where 24-nucleotide-long small interfering RNAs (siRNAs) facilitate methylation of TE sequences leading to heterochromatin formation. Another mode of TE regulation is post-transcriptional gene silencing (PTGS): expressed TEs induce the production of 21 and 22nt siRNAs targeting AGO proteins toward these transcripts. This pathway is also a part of plants' antiviral immunity. 21nt siRNAs mostly lead to mRNA cleavage and degradation, whilst 22nt siRNAs were shown to induce translational repression and, most importantly, recruit RNA-dependent RNA polymerase to the mRNA to produce more double-stranded RNA (dsRNA) leading to amplification of the PTGS.
Duckweeds, or Lemnoideae, small fast-growing aquatic flowering plants, possess a somewhat simplified RdDM lacking many components of this pathway as well as PTGS, missing some elements considered antiviral factors such as AGO2. DICER-LIKE PROTEIN 2 (DCL2), responsible for producing 22nt siRNAs in plants, is also missing in duckweeds, yet we observe 22nt siRNA production in all three species studied in our lab. For instance, transgenic silencing in *Lemna minor* is characterized by the presence of transgene-derived 21/22-nt siRNAs. Transient expression of inverted repeat (IR) in *Spirodela polyrhiza* leads to the production of equal amounts of 21/22-nt siRNAs from the resulting dsRNA. On the other hand, 22-nt siRNAs, together with 21-nt, also come from transposons expressed in *Spirodela*, similarly to *Wolffia brasiliensis.*
The aim of this project is to understand 22-nt siRNA biogenesis in the absence of DCL2 and its biological function in Duckweeds through a combination of genetic and biochemical approaches. Complementation assays in Arabidopsis, knockouts in Lemna's transgenic line and in vitro analysis will be the next steps to investigate this problem.

Keywords: sRNA, PTGS, silencing, plants

*Speaker

Repression of HERV-K negatively impacts astrocyte development

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One of the major challenges towards deciphering the blueprint of life is to understand how functional elements of the genome influence key biological processes. However, the functions of large parts of the human genome including human endogenous retroviral (HERV) elements remain elusive. We could recently show that activation of one specific HERV family, namely HERV-K(HML-2), negatively impacts cortical neuronal development. However, thorough analyses of a larger number of HERV groups are missing to understand their functional contribution to the development of diverse brain cell types beside neurons.
Transcriptomics analysis of post-mortem patient samples demonstrated that HERV groups are differentially expressed between astrocytes and neurons. In particular, we uncovered a substantial upregulation of several HERV-K elements in astrocytes compared to neurons. To unravel the functional contribution of HERV-K to astrocyte development, we applied CRISPR-interference targeting multiple LTRs of the HERV-K group in neuronal progenitor cells. Excitingly, inhibition of HERV-K resulted in a significant decrease in the astrocytic markers GFAP and AQP4 upon differentiation into astrocytes. In contrast, transcriptional repression of HERV-K LTRs had had no effect on neuronal differentiation. HERV-K transcriptional repression also influenced the expression of several cellular genes involved in astrocytic development. In particular, we revealed a C2H2-type ZNF as a promising downstream target, and transcriptional downregulation of the C2H2-type ZNF also resulted in impaired astrocyte development providing mechanistic insight into the identified HERV-K-astrocyte differentiation axis.
In conclusion, our study unveils a previously unknown function of HERV-K in astrocyte development, with implications for brain-specific cellular differentiation. These findings contribute to a deeper understanding of the intricate regulatory mechanisms governing astrocyte differentiation and neurodevelopmental processes.

Keywords: Astrocyte development, HERV, CRISPR interference

*Speaker

The somatic piRNA pathway is progressively activated during embryonic development to effectively repress transposable elements in the adult stage of Drosophila melanogaster.

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Metazoan genomes are largely composed of repeated sequences, including transposable elements (TEs). TEs are DNA sequences that can move from one genomic locus to another. Their mobilization causes genomic instabilities, which can lead to pathologies. In animal gonads, TEs expression is restricted by a specific pathway involving small non-coding RNAs called piRNAs (PIWI-interacting RNAs). These piRNAs target TEs by sequence complementarity, preventing their expression and mobilization.
In *Drosophila melanogaster*, the piRNA pathway is active in adult female gonads: in the germ cells (GCs), but also in somatic gonadal cells (SGCs) surrounding GCs. Some TEs which are abnormally reactivated in SGCs can infect and invade the germline genome. Therefore, activation of the somatic piRNA pathway is also essential for genome protection of the offspring.
The formation of the gonads starts at the embryonic stage. Little is known about the activation of the piRNA pathway and the establishment of TEs repression during development. In our study, we investigated the developmental window during which the somatic piRNA pathway becomes active in the gonads. We monitored the establishment of the repression of TEs, such as *412*, known to be silenced by the *flamenco* piRNA cluster, the major locus involved in piRNA production in SGCs. Our results reveal that *flamenco* and the piRNA pathway actors start to be expressed in SGCs during embryonic gonad formation. Surprisingly TEs are expressed in embryonic SGcs, and progressively repressed later in development, from the larval stage onwards. These results suggest that the somatic piRNA pathway is initiated during embryonic gonad formation and becomes functional during larval stage.

Keywords: Transposable element, small RNA, piRNA, ovary, development

*Speaker

Massively parallel identification of active LINE-1 promoters in human genomes.

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Long INterspersed Element-1 (LINE-1 or L1) forms the only autonomously active family of retrotransposons in humans, and represent 17% of the human genome. L1 retrotransposition is initiated by the transcription of full-length integrated copies driven by an internal promoter located in L1 5' untranslated region (UTR). Furthermore, L1 5' UTR also exhibits antisense promoter activity, which can drive the synthesis of chimeric transcripts with nearby genes. Despite the abundance of these highly repetitive and dispersed sequences, only a limited number of loci are transcriptionally active in any given cell-type and eventually mobilized. This can be explained by the various epigenetic mechanisms that target L1 elements and silence them. However, another non-exclusive possibility is that variations in L1 internal sequence may also affect L1 sense or antisense promoter activities. To identify transcription-competent L1 loci, and test how internal sequence variation affect the activity of individual L1 promoters, we employed massively parallel reporter assays (MPRA) to comprehensively test the sense and antisense promoter activities of human-specific and other recent primate-specific L1 elements (L1HS to L1PA5) present in a given human genome (_˜5000 copies). Taking advantage of the natural variation present in L1 promoter sequences, this strategy will reveal regions and motifs playing important roles in L1 and L1-mediated transcription, as well as transcription-competent loci. This work is supported by Agence Nationale de la Recherche (ANR-11-LABX-0028; ANR-15-IDEX-0001; ANR-21-CE12-0001), Région Sud (MobileGenomAI), Inserm (GOLD Cross-cutting Program on Genomic Variability) and CNRS (GDR 3546).

Keywords: LINE1, L1, 5'UTR, promoter activity, MPRA

*Speaker

Transposable Element Expression During Cardiomyocyte Differentiation

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Transposable elements (TEs) have been shown to play key roles in early mammalian development through acting as regulators of gene expression. However, little is understood about the role of TEs at post-implantation developmental stages, including development of the first organ, the heart. TEs remain notoriously difficult to study due to their repetitive nature hampering efforts to map individual TEs back to their unique loci. TEs are thus often studied in groups of loci, at the sub-family level. Here, we aim to characterise individual TE loci expressed at four stages of cardiomyocyte differentiation, mitigating for difficulties in mapping TEs by making use of the computational framework, TElocal. Secondly, we set out to determine whether TEs characteristic to cardiomyocyte differentiation stages could be acting as regulatory elements for genes governing cardiomyocyte differentiation. Using published bulk RNA-seq and ChIP-seq datasets over the course of cardiomyocyte differentiation, we detect and validate the transient expression of the RLTR4 and ETnERV3-int TE subfamilies, respectively. We also uncover the individual loci contributing to detection of these TE sub-families. Further to this, we find many of the transiently expressed TEs have expression profiles that mirrored peaks observed in H3K4me3 data. This indicates a role for these TE loci as promoters, which regulate the expression of genes involved in cardiomyocyte differentiation. Further insights will be provided with H3K4me1 data, to detect putative enhancers located at TEs which control cardiomyocyte differentiation. Additionally, single-cell long-read RNA-seq data will provide expression profiles of young TEs across cardiomyocyte differentiation which remain largely undetected in this study.

Keywords: Cardiomyocytes, ChIPseq, RNAseq

*Speaker

Nuclear cGAS Defends against Aberrant LINE1 Expression and Stabilizes Genomic Integrity

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Aging-associated inflammation, or 'inflammaging,' is the gradual but significant increase in pro-inflammatory markers in the blood and tissues of aged organisms. This increase in inflammatory markers has been shown to be a hallmark of several age-associated diseases including certain cancers, osteoporosis, arthritis, and kidney disease. Cyclic GMP-AMP Synthase (cGAS) is a cytosolic nucleic acid sensor that functions as a part of the innate immune system to specifically detect double-stranded nucleic acids in the cytoplasm and initiate the proper inflammatory response. While this is intended to target foreign pathogens, endogenous LINE1 (L1) cDNA transcripts, which have been shown to be elevated in aged-mouse models, can also be recognized by cGAS and contribute to misguided pro-inflammatory activity during aging. The unintended consequences of this endogenous immune signaling has led to exploration of cGAS as a potential target to alleviate inflammation and extend healthspan, lifespan, or both. Intriguingly, a significant population of cGAS has also been shown to be stable and inactive when bound to chromatin, but the function of this nuclear localization is largely unknown. Here, we report that cGAS deficiency *in vitro* and *in vivo* results in aberrant L1 mRNA transcription, cytoplasmic L1 cDNA formation, and an increase in pro-inflammatory markers. Additionally, we show that cGAS stabilizes proper genomic organization by repressing L1 transcripts. STED microscopy reveals cGAS resides bound to nucleosomes in heterochromatic regions of the genome, suggesting cGAS prevents L1 expression by repressing transcription. Further, loss of cGAS results in increased susceptibility to DNA damage. These results suggest a previously undescribed regulatory role for nuclear cGAS on repressing L1 elements and stabilizing chromatin organization, suggesting other targets in the cGAS pathway, such as STING, or a specific pharmacological target to the cGAMP producing C-terminus, may serve as more beneficial therapeutic targets in combating inflammaging.

Keywords: cGAS, LINE1, Inflammation, cDNA, nucleosomes

*Speaker

Locus-specific transposable elements in gene expression regulation

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Transposable elements (TEs) comprise about half of the mammalian genome and constitute the most abundant class of non-coding genomic elements known to date. TEs are mostly expressed in the early stages of development and are believed to play roles in recruiting pluripotency-associated factors. Due to their highly repetitive nature, several studies have investigated TEs only within the context of families and subfamilies. However, the challenge with this approach is that it leaves us with little or no insight into the unique role of individual TE loci. This study uses an epigenetic editing technology called iCRUSH to specifically knock down the expression of two candidate TE loci in E14 mouse embryonic stem cells (mESCs) to better understand how individual TE locus regulate nearby genes as well as distal genes critical to cell fate choices. iCRUSH is a doxycycline-inducible piggyBac system that uses an array of GCN4 repeats to recruit up to five KRAB and DNMT3A/3L effectors unto a target locus to induce H3K9me3 deposition and DNA methylation respectively. After iCRUSH targeting of 2 TE loci, we assessed the specificity and reliability of DNA methylation on the specific loci versus other loci with near sequence identity. Furthermore, we measured the enrichment of H3K9me3 by CUT&RUN qPCR of the targeted TE loci. A novel method, CELLO-seq, which uses unique molecular identifiers (UMI) based on single-cell RNA sequencing has been optimised to target TE expression to a particular locus. By utilising CELLO-seq, changes in nearby gene expression levels within 2-10kb were assessed. In the future CELLO-seq will be used to assess whether TEs impact distal pluripotency genes such as *Pou5F1*, *Nanog*, and *Sox2*. This will give us further insight into the role of locus-specific TEs in proximal and distal gene expression regulation.

Keywords: Transposable elements, iCRUSH, CELLOseq, Gene expression, Cell fates

*Speaker

The Plant Mobile Domain proteins antagonize the Polycomb group protein-mediated gene silencing.

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In multicellular eukaryotes, repression of gene transcription by Polycomb group (PcG) proteins is fundamental to cell fate determination and developmental transitions. Cis DNA motifs, called polycomb responsive elements (PREs), allow the recruitment of PcG proteins to their target genes, on which they deposit repressive epigenetic marks such as histone H3 lysine 27 trimethylation (H3K27me3). However, the understanding of the mechanisms controlling PcG recruitment is only fragmentary as certain genes do contain PREs in their promoter sequences, and yet are ubiquitously transcribed implying that they are not targeted by, or refractory to PcG-mediated silencing. This raises the interesting possibility that unknown mechanisms actively protect certain genes from PcG-mediated silencing. The Plant Mobile Domain (PMD) is a protein domain that is widely spread in angiosperms, found associated with transposable elements (TEs) or corresponding to genes playing important roles in the model plant *Arabidopsis thaliana*. The PMD proteins MAINTENANCE OF MERISTEMS (MAIN) and MAIN-LIKE1 (MAIL1) act in a same complex that is required for proper transcription of many genes as well as the silencing of several TEs. Besides, their evolutionary conserved paralog MAIN-LIKE2 (MAIL2) also regulates the expression of several genes. Using complementary approaches combining forward genetics, epigenomics, biochemical and microscopic experiments, we have accumulating evidence showing that the PMD proteins secure the transcription of distinct sets of genes by antagonizing PcG-mediated silencing.

Keywords: Plant Mobile Domain (PMD), Polycomb, regulation of transcription

*Speaker

At the crossroads of epigenetic pathways: H3K27me3 and transposable elements

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The histone modification known as histone 3 lysine 27 trimethylation (H3K27me3) enables transcriptional repression of genes that is both stable across cell divisions and reversible in response to developmental and environmental signals. Despite increasing advances in understanding the mechanisms leading to the establishment of the H3K27me3 mark and its consequences at the gene level, our knowledge of its influence on the inactivation and/or mobilization of transposable elements (TEs) remains extremely limited. In differentiated Arabidopsis somatic tissues, H3K27me3 is present on approximately 10%-15% of TEs. The proportion of TEs targeted by H3K27me3 increases in tissues characterized by natural DNA hypomethylation, such as the endosperm, potentially for their repression. Furthermore, relocation of H3K27me3 marks on TEs is observed in mutants exhibiting DNA methylation defects. Constitutive heterochromatin on TEs appears to prevent the deposition of H3K27me3 and the chromatin state of TEs seems to be monitored by unknown factors that initiate the establishment of H3K27me3 marks in certain specific contexts. To shed light on the intricate molecular interplay between DNA methylation pathways and H3K27me3 deposition at the TEs level, I am employing a combination of genetic and genomic methodologies to address the following question: what chromatin landscape prompts the targeting of TEs by H3K27me3? Previous studies have noted a shift in the localization of the H3K27me3 mark on TEs in *met1* mutant plants. To investigate the impact of non-CG methylation on H3K27me3 relocation at TEs, I have disrupted the RdDM pathway in the *met1* background. The resulting *met1 nrpd2a/2b* mutants display severe phenotypes, including total sterility and homeotic defects, suggesting alterations in H3K27me3 profiles. ChIP-seq analysis of these profiles holds the potential to establish a functional connection between the RdDM and H3K27me3 pathways through yet-to-be-identified mediators. Additionally, integration of methylome and transcriptome analyses of these mutants will offer insights into the underlying mechanisms.

Keywords: DNA methylation, H3K27me3, Arabidopsis thaliana

*Speaker

m6A RNA methylation-dependent regulation of endogenous retrovirus loci

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Transposable elements (TEs) and endogenous retroviruses (ERVs) constitute nearly half of the mammalian genome. TE and ERV activation is critical for normal embryonic development but aberrant expression in later life can cause hemophilia, cystic fibrosis, infertility, metabolic disease, cancer, and neurodegenerative disease. After early development, most TEs and ERVs are fully "silenced" via DNA methylation and histone modifications. However, a subset of ERVs (e.g., certain intracisternal A-particles; IAPs) become variably methylated (VM-ERVs), escape normal silencing mechanisms, and influence neighboring gene expression in *cis* or *trans*, in a cell- or developmental/time-dependent manner. Recent *in vitro* studies indicate that *N*6-methyladenosine (m6A) marks on RNA can regulate gene expression co-transcriptionally or through m6A-methylated nascent RNA interactions with DNA. They showed that decreased RNA m6A levels promotes an open chromatin state and increases regulatory RNA expression (including ERVs). Whether these proposed mechanisms apply to VM-ERV activation/repression remains unknown, yet understanding the epigenetic to epitranscriptomic level of control *in vivo* might help explain the origins of some of the aforementioned diseases. The **objective of this project** is therefore to determine if RNA m6A marks are connected to VM-ERV DNA methylation and VM-ERV activation *in vivo*. This project will provide the first indication that m6A RNA marks regulate VM-ERV activity and VM-ERV DNA methylation state *in vivo*.

Keywords: m6A, RNA, IAP, ERV, methylation

*Speaker

Small RNA Regulation of Endogenous Retroviruses

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Endogenous retroviruses (ERVs) utilize host tRNA as a primer for reverse transcription and replication, a hallmark of long terminal repeat (LTR) retroelements. Their dependency on tRNA makes these elements vulnerable to targeting by small RNAs derived from the 3-end of mature tRNAs (3-tRFs), which are highly expressed during epigenetic reprogramming and potentially protect many tissues in eukaryotes. We found high levels of 3'-tRF in male and female mouse primordial germ cells coincide with elevated ERV burden at sex-specific time points. tRFs are expressed when heterochromatin levels decline, before the onset of piRNA production in males and in the absence of piRNAs in females.

3'-tRFs inhibit ERV activity by blocking reverse transcription but also by post-transcriptional silencing. Due to the perfect sequence complementarity of 3'-tRFs to endogenous retroviral sequences, they have tens of thousands of targets in mammalian genomes. We conducted a massively parallel reporter assay to determine target site rules for 3'-tRFs within the tRNA primer binding site of ERVs. Moreover, we found 3'-tRFs are licensed for *bona fide* RNA silencing by post-transcriptional modifications akin to piRNAs and small RNA classes in other organisms that have perfect sequence complementarity to transposon targets but evade target-directed degradation.

Keywords: ERV, small RNA, tRNA fragment, epigenetics

*Speaker

Reprogramming activates 55 specific polymorphic, functional LINE-1 loci in pluripotent stem cell genomes that were shown to be mobilized in human tumor cells

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We had demonstrated that reprogramming-induced epigenome remodeling in human induced pluripotent stem cells (hiPSCs) resulted in the mobilization of endogenous retrotransposons LINE-1 (L1), *Alu* and SVA, and that intronic L1 *de novo* insertions occuring during reprogramming and hiPSC cultivation, interfere with host gene expression. Here, we set out to identify those specific loci among the _˜1 Mio genomic L1 insertions that encode retrotransposition-competent L1 (RC-L1) elements which are expressed and mobilized in human pluripotent stem cells (hPSCs), causing deleterious insertions. To this end, we generated individualized, custom-tailored genomes of two human embryonic stem cell lines and six hiPSC lines and their parental cells by mapping all fixed and polymorphic RC-L1 loci of these cell lines, and identified 13 fixed and 104 polymorphic RC-L1 loci in these cell lines. To quantify transcripts expressed from the L1-specific promoter of RC-L1 loci, we applied RNA-seq and the 5'RACE method combined with PacBio sequencing, which facilitated mapping of L1-specific RNA reads to unique genomic L1 loci. We mapped those RC-L1 loci that are transcribed in the pluripotent state identifying potential source elements responsible for L1-mediated retrotransposition in hPSCs. Subsets of only 29-63 of the 78-97 cell line-specific polymorphic RC-L1 loci were found activated in hiPSCs relative to their parental cells. One to six specific RC-L1 loci were responsible for _˜50 % of all RC-L1 transcripts. Unexpectedly, 75 of 117 RC-L1 loci identified in hPSCs were shown to be mobilized in cancer cells, and of those, 55 were also expressed in hPSCs. In sum, we uncovered small subsets of 22-54 specific RC-L1 loci that are transcribed and mobilized in each analyzed hPSC line including L1 source loci reported to be responsible for deleterious mobilization events observed in various cancer types. Our findings underscore the significant potential of expressed RC-L1 elements as endogenous mutagens in hPSC.

*Speaker

Keywords: Functional human LINE, 1, Transcriptional activation, Pluripotent stem cells

piRNA clusters adaptability after horizontal transfer of Transposable Element

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On rare occasion, horizontal transfer (HT) of transposable elements (TEs) can occur, threatening the host genome integrity. In gonads, TE activity is suppressed by PIWI-interacting RNAs (piRNAs), a class of small RNAs synthesized by heterochromatic loci enriched in TE fragments, known as piRNA clusters. Maintenance of active piRNA clusters across generations is secured by maternal piRNA inheritance providing the memory for TE repression. Upon insertion of a newly acquired TE into the germline genome, it is commonly believed that the synthesis of specific piRNAs would trigger silencing, thereby ensuring the maintenance of this new TE in future generations. However, the timing of new piRNA emergence remains unclear. To address this question, we used TE-derived transgenes inserted in germline piRNA clusters, along with functional assays and playing with maternal and paternal inheritance of TE-derived transgenes, we have modeled the very first generations after a new TE inserted into a piRNA cluster in *Drosophila melanogaster*. By monitoring the synthesis of new piRNAs across generations, our findings indicate that the complete co-option of these transgenes by germline piRNA clusters can occur within a limited number of generations. Our analysis also revealed heterogeneity of piRNA distribution profile across piRNA cluster loci, never highlighted before. These observations underlie the high potential of adaptation of piRNA clusters, crucial for maintaining genome integrity.

Keywords: piRNA, piRNA cluster, drosophila, germline, Horzontal transfer

*Speaker

Transposable elements biology : from their awakening to their insertion in the genome

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Transposable elements (TE), defined as mobile genomic DNA sequences, are ubiquitously present in all living organisms studied to date. In these recipient organisms, throughout extensive evolutionary periods, hosts and TEs have rather developed specific interactions to facilitate their coexistence and thereby prevent their mutual extinction. In the germline, specific defense pathways are activated by the host to counteract TE expression, ensuring that TE transposition remains low to preserve the host genome integrity and its proper transmission to the next generation. Nevertheless, TEs abundance suggests that they have certainly adapt their modes of expression/integration upon genome colonization, to insure their proper maintenance and propagation.

To explore further these mechanisms, we constructed a *Drosophila melanogaster* line in which the mobility of different TE families can be induced due to the ovarian somatic relief of the piRNA pathway (Barckmann *et al.* 2018, https://doi.org/10.1093/nar/gky761). Thanks to long-read DNA sequencing and a bio-informatic pipeline established in the laboratory (TrEMOLO, https://github.com/DrosophilaGenomeEvolution/TrEMOLO), we precisely determined novel integration sites for five TE families belonging to the endogenous retrovirus group. Interestingly, two of them (ZAM and gtwin) exhibit a dynamic choice of their landing sites. Indeed, we showed that these TEs, to limit competition, have specific expression patterns and integration sites within the host genome. Additionally, we established that their timing of integration during embryogenesis varies.

Unraveling the diverse host-TE interactions that enable this coexistence is a pivotal area of research, that provides valuable insights into the biology of both the host and the genomic parasite like TE.

Keywords: Transposable elements, Drosophila, Integrase, piRNA, Epigenetics

*Speaker

Transposition mechanisms and applications

Transposable element products, functions, and regulatory networks in Arabidopsis thaliana

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Transposable element (TE) mobilization is primarily catalyzed by self-encoded factors, yet these factors have been poorly investigated due to difficulties in defining TE genes in genomes. Here we leveraged extensive long- and short-read transcriptome data, together with structural predictions, to build a comprehensive atlas of TE transcripts and TE-encoded products in the model organism Arabidopsis thaliana. We uncovered hundreds of transcriptionally competent TEs, each of which potentially encoding multiple proteins either through distinct genes, alternatively splicing, or post-translational processing of polyproteins. Structural-based protein analyses revealed dozens of hitherto anonymous domains of unknown functions, and allowed us to build a comprehensive guide of TE-encoded products in Arabidopsis thaliana. Some of these domains displayed significant structural similarity with domains from cellular proteins, suggesting protein co-option or convergent evolution. Furthermore, we identified potential DNA binding and multimerization domains involved in the formation of macromolecular complexes such as transpososomes. In addition, using transcription factor binding site identification and large-scale transcriptome data we demonstrate that TE expression is highly intertwined with the transcriptional network of cellular genes, and identify transcription factors and cis-regulatory elements associated with their coordinated expression during development or in response to environmental cues. This comprehensive atlas of TE-genes and TE-proteins provides a valuable resource for studying the mechanisms involved in the TE-driven evolution of genome function.

Keywords: long read, transcriptomics, functional annotation, protein structure

*Speaker

New determinants of Ty1 LTR retrotransposon integration site selection in yeast

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To maintain host cell survival and ensure their own propagation, many transposable elements (TEs) have evolved the capacity of integrating into specific regions of the genome. This is the case of the Ty1 LTR retrotransposon model of *S. cerevisiae*, which targets regions upstream of Pol III-transcribed genes by means of a direct interaction between its integrase (IN1) and the RNA Polymerase III (Pol III) subunit AC40 (Bridier-Nahmias et al,.2015; Asif-Laidin et al.,2020). Strikingly, the loss of IN1/AC40 interaction redirects Ty1 insertions to subtelomeres, which are gene-poor regions enriched in non-essential stress-responsive genes. While the IN1/AC40 interaction has been delineated at the atomic level (Nguyen et al.,2023), the molecular mechanism of targeted integration at subtelomeric loci has yet to be elucidated. Furthermore, an unexplained mechanism drives Ty1 insertions to two specific hotspots on the nucleosomal DNA (Baller et al.,2012; Mularoni et al.,2012). Altogether, these observations strongly suggest that Ty1 integration pattern is most likely the result of multiple influences, including chromatin structure. To identify novel factors that contribute to Ty1 integration site selection, we first confronted our libraries of Ty1 *de novo* insertion events with the genome-wide distribution of known chromatin features. We observed a striking correlation between Ty1 insertions and the presence of the H2A.Z variant of the histone H2A, suggesting that H2A.Z could contribute to direct Ty1 integration. However, our data indicated that H2A.Z rather prevents Ty1 integration at both *tDNAs* and subtelomeric loci, pointing to a more complex role of H2A.Z in Ty1 integration site selection. In a second approach, we have set up a proximity-dependent biotinylation proteomic screen to identify new partners of Ty1 integrase. By comparing the interactome of IN1 wild-type and loss-of-interaction mutant, we expect to identify common and specific cofactors of Ty1 integration site selection that together contribute to the specific targeting of Ty1.

Keywords: integration, yeast, LTR, retrotransposon, proteomics, chromatin structure, subtelomeres

*Speaker

Benchmarking TE annotation tools for non-model plant species with TEgenomeSimulator

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Transposable elements (TEs) have long been considered repetitive genomic noise that confuse bioinformatics and genomic analyses. TE annotation on a genome assembly has historically been underutilized, more often only applied to hard-masking and filtering out TE sequences before gene annotation. With increasing evidence of both the genetic and epigenetic roles TEs play in gene regulation, environmental adaptation and genome evolution, accurate detection and categorization of these important elements is essential. However, TE annotation tools not only deliver varied results in the representative sequences of TE families and TE categories, but each TE annotator may perform inconsistently across species.
Most TE annotators have been benchmarked on model organisms having manually curated TE libraries for comparison. However, there is no reliable method to assess their performance on non-model organisms. To provide a benchmarking solution for non-model organisms, we integrated and enhanced the approaches used by Wei et al. and Rodriguez and Makalowski, to build TEgenomeSimulator. TEgenomeSimulator is a Python package that can be operated in two modes:

Creating an unstructured synthetic genome with multiple chromosomes, followed by random TE insertions sourced from curated TE libraries;

Utilizing a hybrid approach that inserts TEs randomly into a user-provided non-TE genome where the existing annotated TE features have been removed from a real genome assembly.
In both these modes, curated TE libraries can be mutagenized to simulate varying rates of diversity, e.g., sequence divergence, target site duplications and nested insertion rate, prior to insertion.
To test TEgenomeSimulator, we used the hybrid approach with the recently published assembly of 'Donghong' (*Actinidia chinensis*)7 as an example. We present results from benchmarking a series of tools including EDTA, RepeatModeler2, and EarlGrey on 'Donghong' following insertion of simulated TE sequences generated from curated TE libraries of *Arabidopsis thaliana*, *Oryza sativa*, and *Zea maize*.

*Speaker

Keywords: transposable element, annotation, simulation, benchmarking

Assembly dynamics of the replicative transposition complex of Tn4430 in vitro and in vivo

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Tn4430 is the paradigm of a widespread family of bacterial transposons, the Tn3 family, that are notorious for their contribution of the dissemination of antibiotic resistances. The effectiveness of these elements largely relies on their replicative mode of transposition which allows them to duplicate each time they move within the host genome. Recent genetic, biochemical, and structural studies from our laboratory have led to substantial progress into the understanding of this mechanism. The data support a new model for replicative transposition termed "replication hijacking" according to which transposons integrate into replication intermediates to recruit the host machinery that is necessary for their duplication.
The goal of ma thesis project is to deepen the characterization of this mechanism along two complementary axes. The structural role of the donor and target DNA and the contribution of DNA replication will be studied by exploiting the biochemical assays that I have developed during my Master thesis. The prospect of this part of the project is to reconstruct the full replicative transposition reaction *in vitro*. In parallel, interaction between the transposition partners will be examined in life cells using real-time fluorescence microscopy. Together, the two approaches will provide an integrated view of the "replication hijacking" mechanism *in vitro* and *in vivo*.
Previously, the *in vitro* transposition reaction was performed with simple oligonucleotides that were not really the natural substrates of the transposase. These reactions were carried out with oligonucleotides forming the ends of pre-cleaved transposons and with oligonucleotides forming a replication fork (the best substrate for integration reactions). In this poster, I will present my results showing that transposase can integrate a transposon end found in a non-pre-cleaved plasmid into a replication fork. In addition, experiments showing that transposon flanking sequences in the plasmid can influence integration will be presented.

Keywords: Tn3, Tn4430, Replicative transposition, Electrophoresis, In vitro, Replication hijacking, In vivo

*Speaker

Studying cassette excision dynamics in the sedentary chromosomal integron of Vibrio cholerae

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Integrons are bacterial recombination systems that allow adaptation to environmental stresses and are largely responsible for the emergence and rise of antibiotic multiresistance in Gram-negative bacteria. These versatile systems function by capturing, stockpiling, excising and reordering mobile genetic elements known as cassettes (1). The stable platform of the integron contains an integrase gene (*intI*), and a cassette promoter (Pc) driving the expression of genes encoded in the variable cassette array located downstream of the integration point, the *attI* recombination site. Cassettes consist of promoterless coding DNA sequences (CDSs), associated with a recombination site (*attC*) (2). In this study, we developed a method to access cassette excision dynamics within the model SCI of *V. cholerae* (3) at a population level, using long-read sequencing technology. By taking advantage of the Oxford Nanopore's Read Until technology (4), we achieved a 30,000-fold enrichment of sequencing data precisely targeting the cassettes DNA within the SCI. This high-resolution approach enabled precise measurement of cassette excision rates and facilitated the detection of cassette recruitment events into a synthetic mobile integron. Our dataset will serve as the basis for the development of predictive models, elucidating parameters important for cassette excisions such as the *attC* site sequence, the cassette position in the array, and the size of the cassette. These models will also enable us to predict the likelihood of each cassette being recruited into mobile integrons.

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Keywords: Integrons, Nanopore sequencing, bacteria, cassette dynamics

*Speaker

Cell response to incoming retroviral genomes - Role of the BRCA1/2 DNA repair pathway on the regulation of non-integrated and integrated viral DNAs

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Mobility of retroviral genomes triggers cellular responses that participate in the establishment of stable integration of the virus into the Host chromatin. Viral genome insertion requires the delivery of the integration complex (intasome) to the chromosomal insertion locus followed by the catalysis of integration and post-integrative events. These events include the integration complex disassembly, DNA repair of the insertion site and viral gene transcription. We have previously shown that the DNA homologous repair (HR) processes can modulate both the HIV-1 pre-integrative and post-integrative stages (1–3).
We report here that nuclear foci of the HR RAD51 recombinase are triggered during the early phases of the retroviral infection by a mechanism dependent on the BRCA1/BRCA2 repair pathway. CHiP and imaging approaches indicate that RAD51 is loaded onto the incoming viral DNA as soon as it is synthesized during reverse transcription and before integration into chromatin. Inhibition of this process leads to a decrease in viral infectivity and integration associated with inhibition of reverse transcription and increased persistence of unintegrated viral DNA forms.

Our results thus reveal a new mechanism of cellular response to incoming retroviral genomes participating in regulating the early reverse transcription process, the fate of the different populations of viral DNA and possibly the establishment of persistent and/or latent HIV-1 virus reservoirs.

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*Speaker

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ReactIN SIDACTION grants

Keywords: Retrovirus, integration, integrase, DNA repair, regulation

Revealing Insertion Outcomes of de novo LINE-1 Retrotransposition by an innovative Long-Read Sequencing method

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Long INterspersed Element-1 (LINE-1, L1) is an active protein-coding transposon in the human genome, which can 'copy-and-paste' itself to generate *de novo* genomic insertions using an RNA intermediate; this process is called retrotransposition. Although most genomic copies of L1 (_˜500,000 copies) are immobile, there are approximately 100 loci in human cells that are retrotransposition competent.
Our current working model is that L1 retrotransposition intermediates could result in various insertion outcomes:

- Full-length insertion of L1 (_˜6kb) with target-site duplication (TSD);
- A variant insertion (i.e., 5' truncated/ 5' inverted);
- Trans-mobilisation of small RNA;
- A chromosomal rearrangement (e.g., translocation, duplication and, deletion);
- EN-independent insertion

However, capturing *de novo* L1 insertions in their entirety is technically challenging owing to their varied size, their highly repetitive nature, and their potential association with genomic rearrangements. Currently, **we have very little data on the frequency of each insertion outcome**. In addition, the field has focused on a handful of human cell lines, mainly HeLa in the past few decades, thus, **differences in sequence outcomes across cell lines have not been well characterized**.
To address these technical and knowledge gaps, I have developed a novel Oxford Nanopore long-read sequencing approach to characterize large numbers (> 10,000) of *de novo* L1 retrotransposition outcomes induced by a new retrotransposition reporter in a single sequencing run. I recapitulate known sequence features of L1, various types of L1 insertions, rare L1 events, and L1-mediate genomic rearrangements. Additionally, I revealed that L1 retrotransposition could lead to significantly distinctive insertion outcomes in different cell lines. I anticipate that my data **(1) will reveal complete structures of L1 insertions and provide quantitative metrics of known L1 sequence features and potentially uncover new features**. My work **(2) will shed light on the dynamics of L1 retrotransposition in different cell lines.**

*Speaker

Keywords: long read, sequencing, LINE1, L1, nanopore

Canonical and non-canonical cutting activities of LINE-1 endonuclease

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The apurinic/apyrimidinic (APE)-like endonuclease (EN) of the long interspersed element 1 (LINE-1, L1) initiates retrotransposition by nicking double-strand DNA at a consensus 5-TTTT↓AA sequence, creating a 3-OH group that primes L1 cDNA polymerization through a mechanism termed "target primed reverse transcription" (TPRT). The consensus motif reflects the site of completed retrotransposition events, which depend on A-T base pairing between the cleaved genomic DNA and the L1 RNA poly(A) tail. Therefore, the consensus may not represent intrinsic properties of L1 EN, and it remains unknown whether L1 EN also cuts other sequences. To investigate both canonical and non-canonical cutting activities of L1 EN, we purified monodisperse WT, E43S-D145N double mutant, and D145N-H230A double mutant enzymes to investigate potential non-canonical functions of WT. We have verified conventional nicking activity on a circular plasmid, and demonstrated that the E43S, D145N double mutant ablates cutting activity as expected. Here, we demonstrate a robust, non-canonical cutting activity of L1 EN. Secondary structure analysis and cutting activity on a variety of oligonucleotide fragments reveals that L1 EN favors cutting at positions adjacent to mismatched duplex DNA. This activity may be responsible for aspects of resolving TPRT intermediates, and we plan to expand this study to explore the role of L1 EN in second strand cutting in TPRT.

Keywords: TPRT, Endonuclease, LINE, 1, Retrotransposition, Transposition, Cutting, Genome, Instability, Insertion, Mechanism, Nick, Cut

*Speaker

Host chromatin invasion by retroviral genomes

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Cell chromatin constitutes the first non-return contact point between the genome of incoming infectious agents, as integrative viruses, and their Host. Retroviruses infect both human and animals by integrating their genome into the host chromatin, constituting therapeutic tools, as in gene transfer strategies, and are sources for potential new emerging diseases and zoonoses. The integration of retroviral genome requires the functional association between the viral integration complex (intasome) and the host chromatin involving multiple interfaces between the integrase, the target DNA and the histone components of the nucleosome. These associations are regulated by cellular factors or the structure of the chromatin surrounding the targeted nucleosome. Our project aims to identify these functional interfaces and to analyze the influence of factors regulating integration.
We first characterized the HIV-1 IN-chromatin interactions by biochemical approaches and in a model of chromosomes spreads highlighting IN intrinsic properties of binding to the chromatin and its regulation by its cellular LEDGF/p75 cofactor. We have also shown the importance of both histone tails and the carboxy-terminal CTD domain of IN in this process. Importantly, we demonstrated that the neighboring nucleosomes modulate the functional binding of the intasome to the substrate nucleosome. The use of selected drugs or mutations targeting these interfaces confirmed that they participate in the efficiency of integration but also in the insertion site selection both *in vitro* and in infected cells.

Altogether, these data suggest that retroviral IN CTDs act as sensors of the chromatin structure by scanning available histone and DNA interactions for the selection of functional interfaces for efficient genome invasion.

1. Benleulmi,M.S., *et al., Retrovirology 2017*

2. Matysiak,J. *et al., Retrovirology 2017*

*Speaker

3. Mauro,E. *et al., NAR.*, 2019

4. Lapaillerie,D., *et al., NAR*, 2021

5. Mauro *et al.,* mBio 2023.

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Keywords: Retroviruses, Integration, Integrase, Intasome, Chromatine, Nucleosome

Interplay between the Xer system and the dissemination of antibioresistance in Acinetobacter baumannii

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Antibiotic-resistant (AR) infections pose a pressing challenge in clinical settings. Plasmids are widely recognized for hastening the emergence of AR by facilitating horizontal gene transfer of antibiotic resistance genes (ARG) among bacteria (1,2). To comprehend the dynamics of ARG, it's crucial to grasp the evolution of plasmid strategies geared toward maximizing their persistence and dissemination within bacteria. These strategies may hinge not only on interactions with their bacterial hosts but also on interactions among plasmids themselves.
We explore this inquiry in *Acinetobacter baumannii* (*Ab*), given preliminary evidence suggesting positive interactions between plasmids during the emergence of AR in this bacterium. *Ab* is a globally emerging nosocomial pathogen responsible for a wide array of infections. Genomic scrutiny of the diversity of *Ab* plasmids has revealed that Rep_3 plasmids harbor adaptive genes within variable regions, whose acquisition or loss is believed to involve the Xer recombination pathway. An illustrative instance is the pABV01 plasmid, carrying the *blaOXA24* gene (conferring resistance to carbapenems) flanked by inverted Xer recombination sites (*xrs*). While the backbone of this plasmid closely resembles previously described ones, the region flanked by two *xrs* sites exhibits variability (1). Consequently, it has been postulated that these "*xrs*-cassettes" could constitute a novel mobile genetic element family, mobilized by the conserved Xer recombination system (2,3).

Employing genetic and molecular methodologies, we have tested this hypothesis. Our findings demonstrate that the Xer system of *Ab* is conventional, but "*xrs*-cassettes" do not constitute excisable genetic elements. Intriguingly, we reveal that Xer facilitates recombination between different types of *Ab* plasmids, generating and resolving cointegrate forms. This mechanism elucidates how "*xrs*-cassettes" are exchanged between *Ab*plasmids through interactions among distinct plasmids, offering a fresh perspective for comprehending the dynamics of ARG within bacteria.

1. D'Andrea, et al. (2009). Antimicrobial Agents and Chemotherapy 53, 3528–3533.

2. Balalovski, P., and Grainge, I. (2020). Mol Microbiol. 114(5):699-709.
3. Crozat, et al. (2014). Microbiology Spectrum 2.

*Speaker

Keywords: Site specific recombination, XerCD, Plasmid, Antibiotic, resistance, Acinetobacter baumannii

Fanzor - a cross-domain programmable RNA-guided endonuclease

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Over the past decade, the fascination with programmable RNA-guided DNA nucleases, primarily found in prokaryotes, continues to drive research exploration. Recent discoveries have unveiled a new class of prokaryotic RNA-guided systems known as OMEGA, with the effector TnpB identified as the putative ancestor of Cas12, exhibiting RNA-guided endonuclease activity. Intriguingly, TnpB also appears to share ancestry with eukaryotic transposon-encoded Fanzor proteins, suggesting the potential presence of OMEGA-like programmable RNA-guided endonucleases in eukaryotes. In this study, employing biochemical and molecular biology approaches, we showed that Fanzor proteins use non-coding RNA as a guide to target DNA precisely, and that they can be reprogrammed to edit the genome of human cells. Moreover, the structure of Spizellomyces punctatus Fanzor highlighted the conservation of core regions among Fanzor, TnpB, and Cas12, confirming that Fanzors is a eukaryotic OMEGA system. These results underscored the ubiquitous presence of RNA-guided endonucleases in all three domains of life.

Keywords: Fanzor, RNA, guided endonuclease, eukaryotes

*Speaker

Education and outreach

New REPET improvements: REPET V4.0, making life easier

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The detection and annotation of transposable elements (TEs) are now considered mandatory to any genome sequencing project.

To this aim, the REPET package integrates bioinformatics pipelines dedicated to detect, annotate and analyse TEs in genomic sequences. The two main pipelines are (i) TEdenovo, that search for interspersed repeats, build consensus sequences and classify them according to TE features and (ii) TEannot, which mines a genome with a library of TE sequences, for instance the one produced by the TEdenovo pipeline, to provide TE annotations.
The REPET package is in continuous improvement. Lately our effort have been focused on REPET's ease of use and installation. The new version 4.0 of REPET is now encapsulated in an snakemake framework and uses sSingularity/Apptainer images to handle it's dependency. This allows for seamless integration into HPC cluster environnements.
It enables also an easier code maintenance and evolution, which permit simpler collaborative developement.

Keywords: pipeline, genome annotation, transposable elements, eukaryote, bioinformatics

*Speaker

Developments in Dfam, an Open Community Resource for Transposable Element Families, Sequence Models, and Genome Annotations

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Dfam is an open access database of transposable element (TE) and DNA satellite families, organized around multiple sequence alignments of representative family members ("seed alignments") and their derived consensus and profile hidden Markov models (pHMMs). The initial versions of the database contained meticulously curated transposable element (TE) families and annotations tailored to a select group of model organisms (1,2). Recently, it has undergone significant expansion to accommodate the storage and analysis of a vast array of de novo TE libraries, enhancing its capacity for comprehensive research and exploration. The latest release of Dfam (3.8) has returned the focus to curated libraries contributed by the community with the inclusion of 101 new species libraries, as well as updates to the technologies underpinning the website and API. Moreover, in response to the rapid growth of the database, the FamDB export format has been refactored to support partial downloads of the database partitioned by taxonomic groups. Dfam currently hosts an extensive collection of 3.6 million TE families spanning over 2,437 taxa, and we look forward to its further expansion with your help.
1 Wheeler, Travis J., et al. "Dfam: a database of repetitive DNA based on profile hidden Markov models." Nucleic acids research 2012.
2 Hubley, Robert, et al. "The Dfam database of repetitive DNA families." Nucleic Acids Research 2016

Keywords: Dfam, Database, Community Resource, pHMM, models, API, TE, de novo, curation

*Speaker

Selecting Score Thresholds for Alignment-based TE Annotation with Pseudo-Empirical False Discovery Rates

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The application of transposable element (TE) consensus/pHMM sequence models to genome annotation necessitates the use of alignment score thresholds to manage the level of false discovery. While the success of aligners such as Blast can be attributed, in part, to the development of rigorous sequence alignment statistics, TEs and nucleotide alignment in general pose challenges to their general applicability. We present the methods currently employed by Dfam to generate pHMM thresholds, and recent work to extend this to consensus sequences.

Keywords: Dfam, Sequence Alignment, Thresholding, Benchmarking, Methodology, Evalue

*Speaker

The Somatic Mosaicism across Human Tissues Network: A SMaHT look at Somatic Variation

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The Somatic Mosaicism across Human Tissues Network (SMaHT) is a new NIH Common Fund Program focused on discovering the patterns of somatic variation across the human body, with an emphasis on somatic SNVs, indels, structural variation, and mobile DNA. The NIH Common Fund spurs biomedical advances by seeding new areas of research that require a multi-disciplinary approach and are poised to catalyze future research. The SMaHT network will assess somatic variation in _˜15 post-mortem tissues from a cohort of 150 healthy donors from diverse backgrounds. The goals of SMaHT are to understand how somatic variation affects human biology through: 1. Building a variant catalog of somatic variation in these tissues; 2. Developing innovative tools, technologies, and data pipelines to increase our ability to analyze all types of somatic variation in bulk tissues, as well as single cells and small cellular pools; and 3. Integrating these diverse variant discoveries into a user-friendly SMaHT Data Workbench that can be easily accessed by the broader biomedical research community; and 4. Creating a foundation that future genomic and genetic studies can build on to discover how somatic variation contributes to biological processes in health, disease, and across the lifespan. SMaHT consists of 21 research groups including: 1. A Tissue Procurement Center, which will curate the tissue repository; 2. Five Genome Characterization Centers, which will conduct three core assays (short- and long-read DNA sequencing and RNA sequencing) on all tissues; 3. 14 Technology Development Projects that will improve the ability to accurately detect somatic variation; 4. A Data Analysis Center that will be responsible for ingesting, curating, and analyzing all data from the SMaHT Network; and 5. An Organizational Center that will coordinate SMaHT Network activities and create outreach and engagement strategies for the research community to use these resources. Learn about SMaHT here: https://smaht.org/.

Keywords: somatic mosaicism, human genetics

*Speaker

Miniature inverted repeat transposable elements are enriched in boundaries of topologically associated domains in the carrot genome

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Spatial organization of the genome is hierarchically ordered, ranging from chromosome territories and A/B compartments to more local structures including topologically associated domains (TADs) and chromatin loops. A growing body of evidence suggests that the 3D architecture may affect gene transcription, DNA repair, and replication. In contrast to animal genomes, mechanisms underlying the structural organization of plant genomes remain relatively unexplored.

In this study, we investigated the structure of submegabase-scale domains – TADs in carrot (Daucus carota L.) using publicly available high-throughput chromosome conformation capture (Hi-C) data. Hi-C sequencing reads were processed using HiCExplorer. We identified 239, 477, and 1108 TADs at resolutions of 100 kb, 50 kb, and 10 kb, respectively. In the TAD boundaries we searched for carrot miniature inverted-repeat transposable elements (MITE) copies. We revealed that MITEs were largely enriched at each mapping resolution, on average by 26% as compared to randomly selected genome segments of identical size. Among the MITE superfamilies, namely Stowaways, Tourists, Mutators, and hATs, all groups but Stowaways were significantly enriched. However, the most enriched sequence motif in the TAD boundaries at each resolution, as identified by the XSTREME tool, was precisely the canonical terminal inverted repeat (TIR) of carrot Stowaways. Our findings shed light on structure of TADs in carrot and suggest that MITEs are preferably inserted or maintained within TAD boundaries. Possibly, Stowaways could provide motifs which are important for the TAD formation.

The research was financed by the Polish National Science Center (NCN) (projects Preludium-Bis 2022/47/O/NZ9/00290 and Opus 2019/33/B/NZ9/ 00757)

Keywords: Daucus carota, TADs, MITEs, 3D genome architecture, HiC

*Speaker

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