The role of ORF1p in LINE-1 Retrotransposition

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Abstract

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

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