A comprehensive view on LINE-1 ORF1p granule composition

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Long Interspersed Nuclear Element 1 (LINE-1) remains the only autonomously active retrotransposon in human. Its transcription results in a bicistronic mRNA which encodes two proteins: ORF1p and ORF2p. After translation they preferentially interact in cis with their encoding mRNA and form a ribonucleoprotein particle - an important intermediate for retrotransposition. Besides the endonuclease and reverse transcriptase activities of ORF2p, ORF1p is essential for the process as well, but apart from its nucleic acid binding and chaperone activity its exact roles remain unclear. In cells, ORF1p is mostly compacted within cytoplasmic granules. We aimed to better characterise these granules and, additionally, to map the interactome of ORF1p in living cells in order to better understand the importance of the protein for retrotransposition. Using immunocytochemistry we ascertained that cytoplasmic ORF1p granules differ from stress granules (SGs) as well as from processing bodies (P-bodies). After exposure of cells to different exogenous stressors, however, ORF1p clearly relocates to granules containing bona fide markers of SGs. P-bodies do not overlap with ORF1p granules neither in unstressed nor in stressed cells, although both are frequently in physical contact with one another, especially upon stress. In order to gain a comprehensive view on the composition of ORF1p granules, we used a novel proximity-based labelling method, i.e. biotin identification (BioID), with biotin ligase fused to the C-terminus of ORF1p. Unlike immunoprecipitation, the method enables identification of stable as well as weak and transient protein interaction partners in the environment of living cells. In conclusion, revelation of ORF1p protein network sheds light on known as well as novel potential activators and repressors of retrotransposition and paves the way towards a better understanding of the complexity of the process and its regulation.