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The RD-HRS Method : New insights on the relationship between phase separation, DNA and RNA

The RD-HRS method: new insights on the relationship between phase separation, DNA and RNA

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Recent experimental advances suggest that nuclear bodies dynamically self-assemble and disassemble through condensation and phase separation mechanisms. However, the links between the formation of these nuclear bodies and their association with genomic sequences, and the functional consequences of this association, remain almost entirely unexplored. The difficulty of isolating such semi-liquid structures is a technological barrier that has long prevented in-depth study of their implications for genome architecture and functions.

Our group developed the RD-HRS method (RNA/DNA High-salt Recovered Sequences), a novel experimental approach that allows to purify nuclear bodies by making them insoluble thanks to high-salt treatments of transcriptionally-active cell nuclei. After quantifications of nucleic acids in the insoluble and soluble fractions of HRS assays by quantitative PCR or high-throughput sequencing, we are able to identify not only DNA genomic sequences (Baudement et al., 2018), but also RNAs (Lecouvreur et al., in preparation) associated with them. Using mouse embryonic stem cells, we previously showed that, as expected, the DNA sequences enriched in HRS assays essentially correspond to sequences associated with nuclear bodies (Histone genes/Histone Locus Body; snRNA genes/Cajal bodies; Malat1 and Neat1 genes/speckles and paraspeckles⋯).

Here, using human fetal lung fibroblasts (IMR-90 cells), we performed RT-qPCR experiments on total RNA purified from HRS assays and we show that many, but not all, long non-coding RNAs (lncRNAs) expressed in these cells, like NEAT1, are highly enriched in such RNA-HRS assays compared to messenger RNAs of housekeeping genes. This result emphasizes the important architectural role that lncRNAs play as integral nuclear body components. Our results also indicate that unspliced pre-messenger RNAs of some highly expressed cell-type specific genes, like IGFBP5, are also strongly enriched in the HRS insoluble fraction. Interestingly, both NEAT1 gene and super-enhancers at the IGFBP5 locus are highly enriched in DNA-HRS assays suggesting that not only paraspeckles but also transcriptional condensates are retained in HRS insoluble fraction.

We are now targeting NEAT1 lncRNA using an RNA interference approach in order to inactivate specifically paraspeckles in both proliferative and senescent IMR-90 cells. We will then perform DNA/HRS-seq and HRS/RNA-seq to obtain the first global profiling of DNA and RNA associated with these nuclear bodies. This should bring new insights about the role of paraspeckles in the alteration of genomic organization during senescence.

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