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Poster : RNA profiling of nuclear micro-environments with high molecular crowding

Studying nuclear micro-environments with high molecular crowding, particularly membrane-less organelles (MLOs) such as Cajal bodies, PML bodies, speckles or paraspeckles, has always been a challenge. Indeed, such MLOs typically result from liquid-liquid phase separation and thus behave as liquid-like droplets that are particularly difficult to isolate. The characterization of internal components of these MLOs in different physiological or pathological contexts remains however a key to understand their cellular functions.

We have previously shown that high-salt treatments of transcriptionally active nuclei can make MLOs insoluble, thus allowing to separate them from other soluble nuclear components. This method enables the identification of "High-salt Recovered Sequences"(HRS) as genomic DNA sequences enriched in the insolubilized nuclear micro-environments compared to the soluble fraction. In mouse embryonic stem cells, HRS are associated with the active A chromosomal compartment, including transcription start sites and enhancers of the most highly expressed genes, as well as genes known to be associated with MLOs, e.g. snRNA genes associated with the Cajal bodies, or the Neat1 gene encoding a long non-coding RNAs (lncRNAs) required for paraspeckle formation (Baudement et al., Genome Res. 28:1733-1746, 2018).

We have further improved this method to be able to recover simultaneously the RNA and the genomic DNA from the insolubilized MLOs (RD-HRS method, preprint: https://hal.science/hal-04711849v1). We used IMR-90 cells (human fetal lung fibroblasts) to perform the first global profiling of RNA transcripts associated with MLOs. We found that these transcripts largely consist of specific lncRNAs, some of which known to be associated with specific nuclear bodies, such as NEAT1_2. Moreover, premature RNA transcripts are significantly enriched in the insoluble RNA fraction compared to their mature counterparts. Finally, transcripts with specific intron retention events are also found to be enriched, including one that is dependent on paraspeckle integrity.

We are now combining the RD-HRS method with an RNA interference approach to disrupt paraspeckles and to analyze the RNA and genomic DNA contents of paraspeckles in proliferative and senescent IMR-90 cells.

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