ID de Contribution: **6** Type: **Non spécifié**

RNA profiling of molecular crowed nuclear micro-environments

Studying nuclear micro-environments, particularly membrane-less organelles (MLO) like Cajal-bodies, PML-bodies, speckles or paraspeckles, has always been a challenge. Indeed, such MLO constitute micro-environments with high molecular crowding which typically result from liquid-liquid phase separation. However, the isolation of such liquid-like droplets and the characterization of their internal components is key to understand their roles.

In a previous study, our team showed that such nuclear micro-environments with high molecular crowding can be insolubilized using high-salt concentrations, allowing their isolation from the rest of the nuclear components that remains soluble. It is then possible to identify the so-called "High-salt Recovered Sequences"(HRS) as genomic DNA sequences that are enriched in the insolubilized nuclear micro-environments compared to the soluble fraction. In mouse Embryonic Stem cells, these sequences have been found to be associated with the active A chromosomal compartment, including transcription start sites and enhancers of highly expressed genes, but also with known MLO, like the Cajal-bodies, the speckles and the paraspeckles (Baudement et al. 2018, Genome Research 28:1733-1746).

Here, using an evolution of this method that allows the recovery of RNA in addition to the genomic DNA on IMR-90 cells (human embryonic lung fibroblasts), we performed the first global profiling of RNA transcripts associated with such highly crowded nuclear micro-environments. We found that such transcripts largely correspond to specific long non-coding RNAs (lncRNAs), some of which are already known to be associated with specific nuclear bodies such as Neat1_2, an architectural RNA of the paraspeckles. We also observed that, globally, premature RNA transcripts are more enriched in this insoluble RNA fraction than their mature counterparts. Finally, some transcripts with specific intron retention events are also enriched in such nuclear micro-environments, including one that depends on paraspeckle integrity.

We now plan to combine an RNA interference approach in IMR-90 cells to disrupt the paraspeckles, and then exploit this original way of observing the distribution of RNA in the nucleus to identify the global RNA content, as well as the genomic DNA content of this MLO. In the future, this approach could be extended to a broad range of MLO to better understand their role inside the nucleus.

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Classification de Session: Oral