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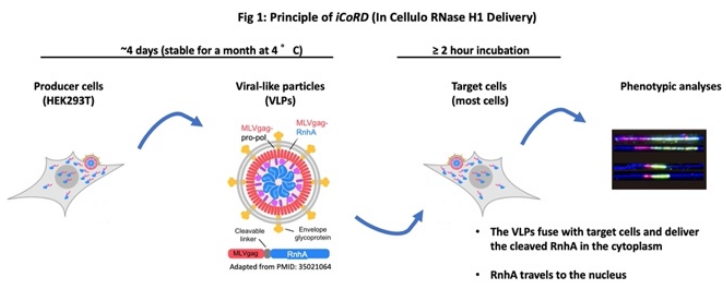
http://www.ens-lyon.fr/LBMC/equipes/architecture-et-dynamique-fonctionnelle-des-chromosomes/Condensin and transcription

Model system: human cells in culture

An innovative strategy to decipher the role of DNA:RNA hybrids in genome instability.

Two fundamental steps are essential for the correct transmission of the genetic material to daughter cells during cell division: (i) the faithful duplication of chromosomes in S phase and (ii) the assembly of chromatin into compact and individualised chromosomes in mitosis. The first of these two steps, DNA replication, is often impacted in pre-cancerous cells. Such replication stress strongly contributes to the genomic instability that characterises many cancerous cells and a key objective of modern research is to understand the direct consequences of such replication stress and how cells respond to it.

DNA:RNA hybrids are physiological by-products of various chromosomal processes. Strikingly, their levels increase strongly upon replication stress and it has recently been proposed that they could serve as diagnostic markers (1). Importantly, the long-term over-expression of RNase H1, an enzyme that degrades DNA:RNA hybrids, partly alleviates the genome instability of cancerous cells, suggesting that DNA:RNA hybrids contribute, directly or indirectly, to this instability and could constitute a promising therapeutic target. However, the precise origin of DNA:RNA hybrids upon replication stress remains unclear. Furthermore, it has been impossible until now to determine the direct consequences of DNA:RNA hybrid formation on the surrounding chromatin because of technical hurdles which prevented the quick elimination of DNA:RNA hybrids from cells.



We have bypassed those technical hurdles by developing *iCoRD* (In Cellulo RNase H1 Delivery), an innovative method that relies on the production of modified viral particles to quickly deliver heterologous proteins in human cells in a very short time (~2h, Fig.1). This strategy is simple and effective and it does not require prior genetic modifications of cells. Moreover, it can be applied to most cell types. Using *iCoRD*, we quickly deliver heterologous proteins that will either remove or stabilize DNA:RNA hybrids. This will enable us to characterize the direct impact of those structures on replication stress. For the first time, *iCoRD* will also enable us to manipulate DNA:RNA hybrids in specific phases of the cell-cycle to better understand when they become toxic.

The internship has two main objectives: (i) to continue optimizing our approach in order to modulate DNA:RNA hybrid formation specifically during early or late DNA replication and (ii) to characterize the impact of such modulations on DNA replication and genome stability. A wide range of techniques will be used: cell culture, production of Viral-Like Particles (VLPs), western blot, metabolic labelling, chromatin spreading, immunofluorescence, FACS. This internship is particularly adapted to candidates considering a future PhD.

Bibliography:

1. Petermann,E., Lan,L. and Zou,L. (2022) Sources, resolution and physiological relevance of R-loops and RNA-DNA hybrids. *Nat Rev Mol Cell Biol*, 10.1038/s41580-022-00474-x.