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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

A new strategy to decipher the putative role of DNA:RNA hybrids in genome organisation.

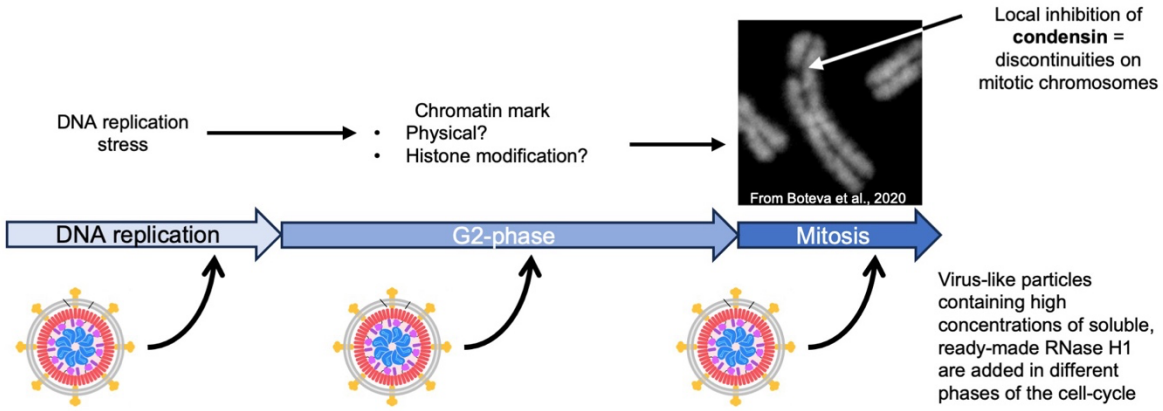

Figure 1: DNA replication stress results in poorly compacted regions on chromosomes at the next mitosis, whose formation can be prevented by increasing the protein levels of RNase H1.

Two main mechanisms 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) in the following mitosis, the condensin-mediated assembly of chromatin into compact and individualised chromosomes in preparation for their segregation to the two daughter cells. The first of these two major steps, DNA replication, is often impacted in pre-cancerous cells. This replication stress strongly contributes to the genomic instability that characterises many cancerous cells and a key priority of current research is to understand the direct consequences of this replication stress and how cells respond to it.

Unexpectedly, replication stress has been shown to have a localized impact on the condensin-dependent assembly of chromosomes in the following mitosis (1). This manifests itself as discontinuities on metaphase chromosomes, corresponding to poorly-compacted regions (Fig.1). A possible model to explain these observations is that replication stress leaves a mark on chromatin that remains throughout G2 until early mitosis, when it inhibits the activity of condensin in *-cis*. This putative chromatin mark could be a physical one (entanglement of sister chromatids) and/or an epigenetics one (dedicated histone modification for example) but its existence and its nature remain elusive (Fig.1).

Interestingly, it is possible to prevent the formation of these discontinuities on metaphase chromosomes by increasing the nuclear levels of RNase H1, an enzyme that degrades DNA:RNA hybrids (2). This observation strongly suggests that DNA:RNA hybrids contribute, directly or indirectly, to these chromosomal discontinuities. It is unclear however whether the extra RNase H1 simply alleviates the replication stress *per se* or whether it plays a more direct role in the condensin-dependent assembly of chromosomes in mitosis. Interestingly, the latter hypothesis is supported by the recently-proposed idea that DNA:RNA hybrids could directly modulate the activity of cohesin, a condensin-related chromosomal complex (see for example (3)).

To determine whether the extra RNase H1 acts during DNA replication or during mitosis to correct the stress-dependent chromosome abnormalities in mitosis, it is essential to be able to deliver this extra RNase H1 in specific phases of the cell cycle. This is not however possible with classical RNase H1 over-expression strategies. We have bypassed this technical hurdle by

developing *iCoRD* (In Cellulo RNase H1 Delivery), an innovative method that relies on the production of modified viral particles to quickly deliver ready-made heterologous proteins in human cells in a very short time (Fig.1). This strategy is simple and effective and it does not require prior genetic modifications of the target cells.

The overall objective of the internship will be to use *iCoRD* to quickly deliver RNase H1 at different times to a synchronous population of cells in order to determine when the *iCoRD*-delivered RNase H1 can efficiently correct chromosome abnormalities in mitosis. Practically, the internship will have three main experimental objectives: (i) to implement the Palbociclib-dependent synchronization of U2OS cells; (ii) to visualize under-replicated regions and discontinuities on mitotic chromosomes and finally (iii) to use *iCoRD* to manipulate the levels of DNA:RNA hybrids in different phases of the cell-cycle. A wide range of techniques will be used: cell culture, production of Viral-Like Particles (VLPs), western blot, metabolic labelling, chromosome spreading, immunofluorescence, FACS. This internship is particularly adapted to candidates considering a PhD in the future.

Bibliography:

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