Poster

New connection between transcriptional regulation and genomic instability

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ABSTRACT

The genes involved in cell and nuclear division ensure an equitable distribution of genetic material between daughter cells. Defects in their functioning cause what is known as genomic instability, a molecular origin in the development of cancer and other genetic diseases.

Among these proteins are crucial those that produce the sister chromatid cohesion and release cycles. This is achieved by the antagonistic action between the loading and unloading pathways of the cohesin complex, which are activated or inhibited in coordination with the progress of the cell cycle, keeping the sister chromatids together until the moment of their separation in anaphase. This regulation is evolutionarily very well conserved and is very similar between multicellular eukaryotes and the fission yeast, Squizosaccharomyces pombe, the model organism in this work. In previous work, the conditional mutant cwf15.d53 was isolated, which has a deletion of the last 53 amino acids that confers sensitivity to formamide and shows an abnormal pattern of mitotic segregation associated with a quantitative and qualitative increase in chromosome-loaded cohesin.

The aim of the project is twofold: on the one hand, to characterise a new thermosensitive allele of cwf15 (cwf15.ts4) and, on the other hand, to determine if this is the molecular cause of this segregation defect. The approximation we follow in the first case is to reveal the cellular defects caused under restrictive conditions, by fluorescence microscopy in live and fixed cells. To deal with the second objective, we expect to over-express one of the proteins involved in cohesin unloading, Wpl.

To do this, its promoter at the native locus will be replaced by a powerful regulatable promoter (nmt promoter). Its expression is regulated by the amount of thiamine present in the medium, so that in the absence of thiamine it is highly expressed. In this situation, we can check if the cellular defect is reversed and monitor the amount of cohesin throughout the cell cycle by biochemical assays and fluorescent markers in live cells.

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