Séminaire externe

"Genome-wide distribution of RNA-DNA hybrids identifies RNase H targets in tRNA genes, retrotransposons and mitochondria."

During transcription the nascent RNA can invade the DNA template, forming extended RNA-DNA duplexes (R-loops). These structures are potentially deleterious for gene expression and genome stability, but can be beneficial, for example, during immunoglobulin gene class-switch recombination in B cells.

We have made use of antibody S9.6, with specificity for RNA-DNA duplexes independently of the sequence, to map genome-wide the distribution of RNA-DNA hybrids in Saccharomyces cerevisiae. As expected, R-loops in the wild-type were strongly associated with the highly transcribed RNA polymerase I (Pol I) ribosomal DNA (rDNA) genes. R-loops were also enriched over highly expressed protein-coding genes, which are transcribed by Pol II, particularly the second exon of spliced genes. Conversely, intron regions of spliced genes were depleted of R-loops. We speculate that R-loops are suppressed at intron regions to ensure proper recognition of 5' and 3' splice sites by the splicing machinery, whereas they are favoured over exon 2 to decelerate elongation of Pol II and by doing so to promote co-transcriptional splicing.

On Pol III loci such as the highly transcribed tRNA genes, R-loop accumulation was strongly detected in the absence of both RNase H1 and H2, indicating that R-loops are inherently formed but rapidly cleared by RNase H. Importantly, accumulation of stable R-loops at tRNA genes in mutants lacking both RNase H and DNA topoisomerase I (Top 1), or also Top2, lead to reduced synthesis of tRNA precursors. This is reminiscent with stable accumulation of R-loops in these mutants at the rDNA which leads to reduced synthesis of pre-rRNA precursors. RNA-DNA hybrids were strongly associated with Ty1 cDNA retrotransposition intermediates in the absence of cellular RNase H, and this was accompanied by increased Ty1 retrotransposition, particularly at tRNA genes. Pol III-associated-R-loops may favour insertion of Ty1 elements by integrase-mediated integration, at nucleosomes located upstream of tRNA genes. It is also possible that DNA damage inflicted on the nuclear genome by R-loop accumulation, in particular in mutants lacking both cellular RNase H and Top1, leads to the alleviation of Ty1 retrotransposition dormancy.

Finally, R-loops were strongly associated with yeast mitochondrial transcription units and were specifically cleared by nuclear RNase H1 but not RNase H2.

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Salle de conférence du CBM