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Master Project Proposal

Title: Investigating R-loops as regulators of splicing

Synopsis:

R-loops are three stranded nucleic acid structures that form co-transcriptionally, when an RNA hybridizes with the complementary template DNA strand, forming a DNA/RNA hybrid and a displaced single stranded DNA (ssDNA)[1]. Although these structures are often seen as sources of genomic instability, R-loops are also emerging as powerful regulators of several processes, such as gene expression, class-switch recombination, and telomere stability. R-loops have both beneficial and deleterious roles. They serve as binding site for factors regulating chromatin states, epigenetic modifications, transcription initiation and termination, antibody diversification and DNA repair. However, conflicts between R-loop-stalled transcription complexes and replication forks are a potent source of DNA damage and threaten genome stability[2].

Splicing is the co-transcriptional removal of introns from the pre-messenger RNAs (pre-mRNAs) and is a versatile means of genetic regulation that is often disrupted in human diseases[3]. Surprisingly, R-loops have never been shown to directly impact or regulate splicing, even though most human genes are alternatively spliced, and R-loops may form genome wide in virtually any gene. Nevertheless, previous data raise the hypothesis that such a role may indeed exist. For instance, several splicing factors are known to interfere with R-loop formation, R-loops form frequently in introns and G/C-rich DNA sequences, known to favour R-loop formation are enriched upstream of weak 3' splice sites (3'SS) in non-canonical introns[4,5]. Our data indicate that R-loop formation juxtaposed upstream a weak 3' splice site increases the splicing of that intron. Altogether, these findings support our hypothesis that R-loops can directly interfere with splicing.

In this project we aim to investigate how R-loop formation affects splicing. To achieve this, we generated splicing reporter systems together with tools to efficiently remove R-loops which allows us to directly address the impact of R-loops on splicing. We will use molecular biology techniques to determine splicing efficiencies and fluorescence microscopy to observe our tools to remove R-loops in live cells.

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