The Second Life of Intron

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In mammalian genomes, coding sequences (exons) are intervened by long non-coding sequences (introns) which compose ~90% of genes. Pre-mRNA splicing is a remarkable process to remove long introns and ligate exons. The intron is excised out from a gene in a lariat shape with a unique 2'-5' phosphodiester bond at the branchpoint. Degradation of the long intronic lariat is essential to recycle and release the nucleic acids, splicing machinery and RNA binding proteins. Debranching enzyme DBR1 specifically hydrolyzes the 2'-5' phosphodiester bond, exposing ends of the introns for further degradation. Because of the transient nature of the RNA lariat, the specificity of DBR1 is poorly studied.

By biochemical enrichment of DBR1-senstive lariats and computational selection of deep sequencing reads that traverse branchpoints, we demonstrate that in human cells, DBR1 catalytic activity prefers short introns over long introns, A-branchpoint over C-branchpoint. Moreover, the upstream and downstream sequences to the branchpoint also affect DBR1 recognition. These results suggest some introns are more dependent on DBR1 and others may require other nucleases for turnover. Interestingly, the best DBR1 catalytic substrate is the most robust branchpoint sequence for splicing, suggesting that DBR1-mediated intron turnover co-evolves with the branchpoint splicing activity. Introns that escape DBR1 digestion in the nucleus get transported to the cytoplasm. They are shorter, GC-rich and contain mostly C-branchpoints. AU-rich introns that escape the nucleic degradation are re-ligated into stable intronic circles by a post-splicing reaction. These stable circular introns may have cellular functions as molecular sponges or transcription factors. Finally, we demonstrate that the branchpoint position and debranching direct the maturation of intron-coded CD-box snoRNAs. Over all, we show that introns with different length, GC-content and branchpoints, turn over through differential pathways, and that the regulation of intronic lariat hydrolysis is not only critical for recycling nucleic acid and protein factors but also for regulating several cellular processes.