ALTERNATIVE SPLICING

Scrutinizing spliceosomes

" new approaches to isolate and interrogate intronic sequences from yeast spliceosomes "

The inclusion or exclusion of introns is an important regulatory stage in gene expression, affecting the structure, function and amount of protein product. Two articles published in *Cell* describe new approaches to isolate and interrogate intronic sequences from yeast spliceosomes, providing a transcriptome-wide picture of intron diversity and shedding new light on the process of splicing.

Until now, annotation of introns has relied mainly on indirect data, such as sequences absent from mature mRNA transcripts or computational prediction based on various assumptions, including the presence of conserved 3′ and 5′ splice sites (SS) and the branch point (BP). Opportunities to directly examine the normal process of splicing have been rare. Now, Chen et al. and Burke et al. report methods for high-throughput sequencing of intronic RNA contained within endogenous spliceosomes.

Chen and colleagues capitalize on previous work in which they purified *Schizosaccharomyces pombe* spliceosomal complexes used in the final stages of splicing, when they contain predominantly branched (lariat) intron products. Now, the team describe a deep sequencing approach to fully profile the sequences contained within this complex by use of multiple library types, including spliceosome RNA sequencing (RNA-seq) libraries (to map all associated RNAs), 5′-P libraries (to map intron 5′ ends) and 3′-OH libraries (to map intron 3′ ends). By incubating spliceosomes with micrococcal nuclease before RNA purification, the authors generated spliceosome 'footprints', thus also mapping intron–spliceosome interactions.

Credit: Martin Konopka/EyeEm/Getty

The team identified more than 200 new introns and corrected the sequences of several misannotated introns. In many cases, the spliced products were not present in RNA-seq data, presumably being promptly degraded by nonsense-mediated decay, which emphasizes the limitations of indirect methods for intron discovery.

The team identified examples of unusual processes such as recursive splicing, whereby introns are removed in multiple steps rather than as single units. The study also revealed extensive spliceosome footprints located around the SS and BP, as well as a third footprint in longer introns upstream of the BP. The authors propose that this region binds Cwf11, the *S. pombe* homologue of the RNA helicase Aquarius, which forms an important part of the human spliceosomal C (catalytic) complex. Finally, the team developed a new method for mapping BPs.

Burke and colleagues developed a suite of similar methods, which they applied to three different yeast species. The authors used affinity purification of the splicing factor Prp19, which is associated with actively-splicing and post-catalytic complexes, followed by high-throughput sequencing to compare the spliceosome profile — including 3′ ends, junctions and branch profiles — of the intron-reduced but experimentally-tractable *Saccharomyces cerevisae* with two intron-rich fungi: *S. pombe* and the more distantly related human pathogen *Cryptococcus neoforma*. The team were able to map canonical and non-canonical splicing events, including interrupted, recursive and nested splicing, in which a smaller intron is excised from a larger intron.

Using a quantitative modelling approach on the two intron-rich species, the authors showed that strength and spacing of the 5′ SS and BP sequences were predictors of the amount of spliceosome-bound precursor and intermediate substrate levels.

Intron size is thought to be under strong evolutionary pressure; although intron size in the studied yeasts is variable, Burke et al. found that longer introns tended to have stronger splicing signals, presumably to mitigate inefficiencies associated with length. Moreover, transcripts with many introns tended to have lower levels of bound intermediate, raising the intriguing suggestion that spliceosomes on the same transcript are interacting with each other.

Burke et al. then focused on a process termed 'discard', in which premature spliceosome disassembly occurs when (presumably low-quality) pre-mRNAs are slow to undergo splicing. The authors were able to show that spliceosome discard promotes intron retention and frequently occurs in vivo.

In analogy to ribosome profiling, which has sparked great advances in our understanding of post-transcriptional gene regulation, both groups refer to their approaches as 'spliceosome profiling'. Importantly, these approaches can be tailored to elucidate many other poorly understood aspects of the splicing process.

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ORIGINAL ARTICLE Chen, W. et al. Transcriptome-wide interrogation of the functional intronome by spliceosome profiling. *Cell* **173**, 1031–1044 (2018) | Burke, J. E. et al. Spliceosome profiling visualizes operations of a dynamic RNP at nucleotide resolution. *Cell* **173**, 1014–1030 (2018)

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