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RNA SPLICING

Counting, coordinating and controlling the alternatives

> “[…] the extent of tissue-specific regulation of mRNA processing is far greater than previously estimated.”

Alternative splicing of precursor mRNA is a key mechanism for generating proteomic diversity. Two new studies using high-throughput sequencing technology now indicate that the vast majority of human genes have multiple splice forms. Together with two other recent studies, which have also used transcriptome-wide analysis, this research gives new insights into how tissue-specific splicing might be regulated.

Deep sequencing of cDNA from multiple human tissue types by Wang, Pan and colleagues revealed thousands of new splicing junctions. Both studies conclude that approximately 92–98% of human multi-exon genes (at least 86% of all human genes) are subject to alternative splicing. Wang *et al.* identified over 22,000 tissue-specific alternative transcript events — including alternative splicing, alternative polyadenylation (APA) and alternative promoter usage — which suggests that the extent of tissue-specific regulation of mRNA processing is far greater than previously estimated. Correlation between the tissue-specific patterns of alternative splicing and APA events also led the authors to hypothesize that these two mechanisms of alternative mRNA processing might be coordinately regulated.

In an independent study, Licatalosi and colleagues found evidence that a tissue-specific RNA-binding protein (RNABP) is involved in regulation of both splicing and polyadenylation. By coupling immunoprecipitation of RNABPs crosslinked to RNA fragments with high-throughput sequencing (a technique called HITS–CLIP) they produced a transcriptome-wide map of RNA-binding sites for the neuron-specific splicing factor Nova in the mouse brain. The pattern of binding sites suggested that Nova might regulate APA as well as alternative splicing, and this was confirmed by functional studies, including studies in *Nova2* knockout mice. Together, the Wang and Licatalosi studies point towards tissue-specific regulatory proteins as the link between different aspects of alternative mRNA processing.

Both papers also highlight sequence motifs recognized by RNABPs as crucial determinants of RNA-processing regulation. On the same theme, another recent study, by Castle *et al.*, identified 143 motifs associated with variation in alternative splicing between tissue types. To screen for these motifs they looked for nucleotide ‘words’ that were under or overrepresented near splice sites, based on a large compendium of splicing events compiled by whole-transcript microarray analysis of samples from 48 human tissue and cell lines.

All these studies have used recently developed technological tools to explore the regulation of RNA processing and have added to the understanding of the extent and complexity of control at the transcript level. The coordination of splicing with polyadenylation might ensure that the correct elements are included in both the regulatory and coding regions of tissue-specific transcripts. Mapping the pattern of RNABP binding to the newly identified sequence motifs, using HITS–CLIP, might help to explain how some of the vast array of alternative transcripts described in these studies are expressed in the appropriate tissues.

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ORIGINAL RESEARCH PAPERS Wang, E. T. *et al.* Alternative isoform regulation in human tissue transcriptomes. *Nature* 2 Nov 2008 (doi:10.1038/nature07509) | Pan, Q. *et al.* Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nature Genet.* 2 Nov 2008 (doi:10.1038/ng.259) | Licatalosi, D. D. *et al.* HITS–CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* 2 Nov 2008 (doi:10.1038/nature07488) | Castle, J. C. *et al.* Expression of 24,246 human alternative splicing events and predicted *cis* regulation in 48 tissues and cell lines. *Nature Genet.* 2 Nov 2008 (doi:10.1038/ng.264)
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