*Nature Reviews Genetics* | AOP, published online 6 December 2011; doi:10.1038/nrg3137

GENE EXPRESSION

# Splicing steps aside to consider its options

Splicing of precursor mRNAs generally occurs co-transcriptionally, thus promoting the correct sequential splicing of exons as they emerge from RNA polymerase II. However, for alternatively spliced exons, deferring splicing until all of the optional exons have been synthesized would be beneficial. A new study uses single-molecule transcript visualization to show that the splicing of alternative exons can be temporally and spatially uncoupled from transcription and that this can involve sequestering splicing signal sequences either in RNA secondary structures or by protein binding.

Tyagi and colleagues applied fluorescent hybridization probes to track splicing events; the use of up to hundreds of fluorophores amplified the signals and allowed the visualization and counting of single transcript molecules.

The authors studied a *GFP* transcript with two engineered introns — consisting of canonical splicing signal sequences and distinct probe-binding sites — in Chinese hamster ovary cells. They probed the 3′ untranslated region (UTR) to localize all *GFP* transcripts and monitored the progression of splicing through the loss of colocalization with the intronic probes. They found that one of the introns was always spliced out co-transcriptionally but that splicing of the other was uncoupled from transcription so that this intron was retained in the transcript throughout the nucleoplasm. This effect was independent of the order of the introns in the construct, indicating that uncoupling from transcription can be an intrinsic feature of an intron.

What allows the escape of an intron from co-transcriptional splicing? RNA secondary structure predictions showed that the polypyrimidine tract of the uncoupled intron was sequestered in a hairpin structure and was therefore probably unavailable to bind splicing factors such as U2AF. This sequestration can be sufficient for uncoupling splicing from transcription: altering sequences near the polypyrimidine tract in a co-transcriptionally spliced *FOS* gene intron to generate secondary structure uncoupled its splicing from transcription.

The authors extended their analyses to transcripts in which alternative splicing by exon skipping is mediated by transcript-binding proteins that display context-dependent expression patterns. Specifically, they looked at the effects of removing Sex lethal (SXL), a female-specific protein in *Drosophila melanogaster*, or the polypyrimidine tract binding (PTB) protein, which is specific to mammalian non-neuronal lineages. The absence of these proteins — of SXL in male *D. melanogaster* cells or of PTB by RNAi in HeLa cells — not only resulted in the expected shift from alternative to constitutive splicing of the relevant exons in the target transcripts but also caused a recoupling of splicing and transcription.

Overall, this study highlights that cells have the ability to choose the most suitable splicing strategy for different exons within individual transcripts. Additionally, it shows that sequestering particular transcript sequences can convert splicing from being constitutive and co-transcriptional to being alternative and transcriptionally uncoupled. It will be interesting to characterize the molecular mechanisms of the uncoupling and to uncover to what extent these splicing principles apply across entire transcriptomes.

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**ORIGINAL RESEARCH PAPERS** Vargas, D. Y. *et al.* Single-molecule imaging of transcriptionally coupled and uncoupled splicing. *Cell* **147**, 1054–1065 (2011)