GENE EXPRESSION

# RNAPII stands alone

> “elongation rates are uncoupled from splicing.”

The idea that mRNA splicing occurs co-transcriptionally makes sense given the long lengths of many eukaryotic genes. Furthermore, it has become increasingly apparent that splicing is itself regulated by transcription, as the rate of polymerase elongation can influence the regulation of alternative exon inclusion. Now, Brody *et al.* turn this idea around and ask how splicing influences polymerase elongation. Surprisingly, they find that the relationship between RNA polymerase II (RNAPII) elongation kinetics and splicing is not reciprocal: elongation rates are uncoupled from splicing.

To allow them to compare transcription rates between genes with varying numbers of introns, the authors generated a series of constructs based on the human β-globin gene. The modified gene contained a tetracycline-inducible promoter, so that the gene could be turned on at will, and an array of MS2 sequence repeats, which form stable RNA loops that bind yellow fluorescent protein (YFP)-tagged MS2 to allow the visualization of mRNA. To this basic gene they added copies of the same exon and intron, resulting in constructs with no introns, two introns or three introns. These were co-transfected into human cells with a plasmid that contained multiple copies of the *lacO* repeat, to allow determination of the integration site.

This enabled them to study the kinetics of transcription in live cells through three-dimensional fluorescence recovery after photobleaching (FRAP) analysis. This used the signal from red fluorescent protein–LacI (which binds *lacO*) to locate the site of transcription, and tracking of the YFP–MS2 signal recovery after photobleaching to estimate the rate of mRNA accumulation. They found that recovery rates of the YFP–MS2 signal were the same, regardless of the number of introns. Thus, in this system, splicing does not interfere with polymerase elongation rates.

Interestingly, when the authors extended their analysis by adding two extra copies of the same intron and exon to the construct, the signal recovery rate of YFP–MS2 was reduced. However, the rate of RNAPII accumulation after photobleaching (followed using green fluorescent protein-labelled RNAPII) was the same for all four constructs, indicating that there was no polymerase blocking or pausing during elongation. Instead, their data suggest that the delay in MS2 signal recovery is due to the retention of mRNA at the site of transcription.

This suggests that when transcription has completed but splicing has not, the splicing machinery is retained at the site of transcription, independently of polymerase. Thus, elongation rates are independent of ongoing splicing, and splicing can occur post-transcriptionally while the pre-mRNA is still tethered to the chromatin.

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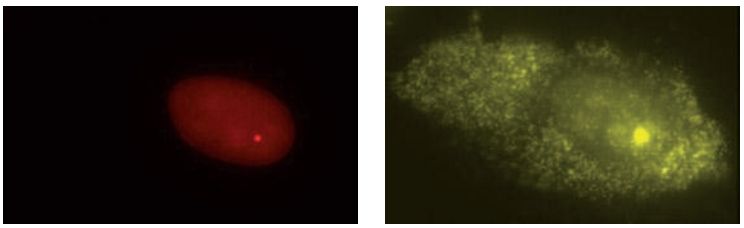

Image shows the gene locus, labelled with red fluorescent protein–LacI (left), and the transcribed mRNA, labelled with yellow fluorescent protein–MS2 (right), at the site of transcription and throughout the cell. Image is modified from Brody, Y. *et al.*

**ORIGINAL RESEARCH PAPER** Brody, Y. *et al.* The *in vivo* kinetics of RNA polymerase II elongation during co-transcriptional splicing. *PLoS Biol.* **9**, e1000573 (2011)