Nature Reviews Molecular Cell Biology | AOP, published online 12 March 2014; doi:10.1038/nrm3774

Jim Zuckerman / Alamy

TRANSCRIPTION

Splicing keeps RNA polymerase II in check

> RNAPII stalling in *prp5-1* cells is Cus2p-dependent.

Splicing of primary mRNA transcripts occurs co-transcriptionally, but it is unclear to what extent splicing efficiency affects transcription. Beggs and colleagues report that in conditions that block pre-spliceosome complex formation in *Saccharomyces cerevisiae*, RNA polymerase II (RNAPII) stalls on introns in a Cus2p-dependent manner, which suggests the existence of a splicing-associated transcription elongation checkpoint.

The formation of pre-spliceosome complexes requires the spliceosomal RNA helicase Prp5p and is facilitated by the U2 small nuclear ribonucleoprotein (snRNP)-associated protein Cus2p, which is then displaced to enable splicing to proceed. Yeast cells with the heat-sensitive *prp5-1* mutation fail to displace Cus2p when shifted to the restrictive temperature and are defective in pre-spliceosome formation. To test whether this might affect transcription, the authors performed chromatin immunoprecipitation (ChIP) analysis using RNAPII-specific antibodies along the length of several genes in *prp5-1*-mutant cells. Shifting mutants to the restrictive temperature resulted in RNAPII accumulation on introns of intron-containing genes but not on intronless genes. RNAPII accumulation at introns was not observed when a different stage of splicing was disrupted, which indicates that it is not a general consequence of defective splicing. Interestingly, the accumulated RNAPII was specifically phosphorylated at Ser5 of its carboxy-terminal domain, which indicates a possible transcription elongation defect in the *prp5-1* strain. Next, the authors carried out a genome-wide ChIP followed by sequencing analysis of the *prp5-1* strain and found widespread RNAPII enrichment on introns and depletion from downstream exons, which further suggests a defect in transcription elongation.

As the requirement for Prp5p to form a pre-spliceosome complex is reduced in the absence of Cus2p, the authors deleted *CUS2* from the *prp5-1* strain and found for several genes that RNAPII distribution was similar to that in wild-type cells. Reintroducing *CUS2* restored RNAPII accumulation on introns at the restrictive temperature, which confirms that RNAPII stalling in *prp5-1* cells is Cus2p-dependent.

To confirm a defect in transcription elongation in the *prp5-1* strain, the authors measured 4-thio-uracil incorporation into newly synthesized transcripts. They found that the amount of RNA produced in the labelling period from intron-containing genes, but not from intronless genes, was greatly reduced in the *prp5-1* mutant strain compared with wild type cells. Furthermore, deletion of *CUS2* rescued this defect but not the splicing defects of *prp5-1* mutant cells. So, it seems that the *prp5-1* mutation reduces the amount of nascent RNA of intron-containing genes in a Cus2p-dependent manner and not simply as a consequence of a splicing defect.

On the basis of their results, the authors propose the existence of a splicing-dependent transcription elongation checkpoint, which is triggered by Cus2p if pre-spliceosome formation is unsuccessful. Interestingly, Cus2p is the putative orthologue of human TAT-specific factor 1 (TATSF1), which is associated with both RNAPII and U2 snRNPs. Whether the dual functions of TATSF1 in transcription and splicing are coupled remains to be investigated.

Eytan Zlotorynski

ORIGINAL RESEARCH PAPER Chathoth, K. T. *et al.* A splicing-dependent transcriptional checkpoint associated with prespliceosome formation. *Mol. Cell* **53**, 779–790 (2014)