Please hold—the next available exon will be right with you

Karla M Neugebauer

A major challenge in gene expression is to understand how precursor messenger RNA (pre-mRNA) processing events are integrated with transcription. A recent study suggests that distant exons in nascent RNA are held together during transcription, promoting accurate splicing independent of intron fate.

A recent paper by Dye *et al.*[1] shows that pre-mRNA splicing occurs efficiently in cells even when introns have been cleaved by an inserted ribozyme or cotranscriptional cleavage element (CoTC). In other words, the pre-mRNA need not be a continuous linear molecule for the exons to be properly matched and ligated together, even in the context of the living cell nucleus, where heterogeneous RNA molecules abound.

Why is this surprising? First, we know that a naked, intron-containing pre-mRNA can be spliced *in vitro*; the splicing reaction depends on assembly of spliceosomal small nuclear ribonucleoprotein particles (snRNPs) and non-snRNP factors, for the most part, on regions of the pre-mRNA that are close to the 5′ and 3′ splice sites[2]. The linearity of the pre-mRNA substrate would seem to ensure proximity of the 'reagents' in the two-step transesterification: the branchpoint, the 5′ splice site and the 3′ splice site (**Fig. 1**). However, we also know that separately synthesized RNAs harboring 5′ and 3′ splice sites can be '*trans*-spliced' together *in vitro*[3,4]. Thus, colinearity of 5′ and 3′ splice sites is not strictly required for splicing. However, if *trans*-splicing were a frequent occurrence in cells, exons from different transcription units would be erroneously spliced together, producing proteomic chaos. Indeed, an experiment designed to detect *trans*-splicing of cleaved pre-mRNAs in the present study proves that *trans*-splicing between different transcripts does not occur here. It would seem logical that the integrity of each pre-mRNA should ensure that exons from each transcript keep to themselves.

In vivo, pre-mRNA splicing begins cotranscriptionally, while the nascent RNA is still attached to DNA by RNA polymerase II (Pol II). Extrapolating from *in vitro* splicing, one could thus imagine the nascent RNA as a springy phone cord extending from the transcription machinery, with nascent 5′ splice sites flapping in the nucleoplasmic breeze while the rest of the intron and the next exon are ploddingly synthesized. This vision is encouraged by classic images of lampbrush chromosomes, in which nascent RNA splays out from the DNA axis on glass or an EM grid. In particular, evidence that splicing is cotranscriptional originates from studies of Miller chromatin spreads, with RNPs detected on hair-like RNA projections from the DNA and with shortening of nascent RNA owing to splicing before transcription termination[5]. However, these preparations are specifically designed to spread chromatin and nascent RNA for the purpose of analysis, and no one ever claimed that nascent transcripts would flap in the breeze *in vivo*.

A key observation was provided by electron tomography of Balbiani ring genes of *Chironomus tentans*[6]: nascent RNPs lie adjacent to the DNA axis, like currants along a common stem. They contain Pol II as well as the U2 snRNP, which are bound to distant points on the pre-mRNA, suggesting that the nascent RNA is wound up with associated proteins upon exiting the polymerase (**Fig. 1**). However, results from this imaging technique do not exclude the possibility that strands of nascent RNA loop in and out of the nascent RNP, and they leave open the question of how nascent RNA is arranged during transcription and RNA processing. The work of Dye *et al.*[1] now strongly supports the argument that at least the exons must be held in proximity in such nascent RNPs, so that upstream exons do not float away before the next exon is synthesized. Data indicating that intron cleavage is cotranscriptional and precedes splicing are essential for this conclusion. Indeed, selection of nascent RNAs produced in a nuclear run-on assay shows that the CoTC-containing intron is cleaved before the next exon is synthesized (**Fig. 1**). Parallel experiments with constructs containing a ribozyme in the intron are less definitive: reverse-transcription PCR of total RNA indicates that the intron is cleaved to completion; however, as the primers used are located within the intron itself, some of the cleavage could conceivably have occurred after splicing.

The authors conclude that it must be Pol II itself that holds upstream exons; however, no experiment in the study addresses this directly. In fact, it is quite possible that nascent RNPs form quickly, wrapping up the RNA in a

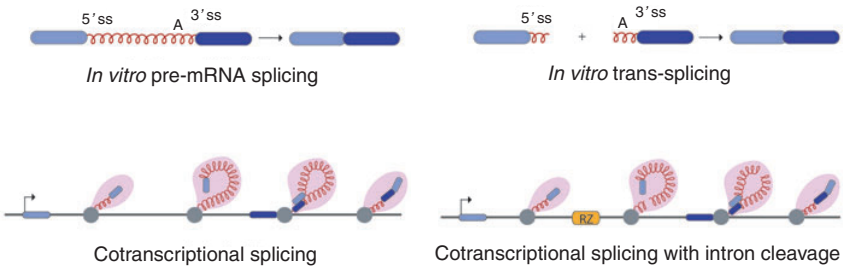

Figure 1 Pre-mRNA continuity in splicing. Schematic diagrams show pre-mRNA as a continuous molecule (left) that serves as a substrate for *in vitro* splicing and *in vivo* cotranscriptional splicing. Discontinuous pre-mRNA substrates (right) can be spliced *in vitro* by *trans*-splicing or *in vivo* by retention of the nascent RNA within nascent RNPs. The orange segment labeled RZ represents the introduction of a cleavage site in the intron, either a ribozyme or the CoTC element. Intron cleavage indicated is based on the experimental results of Dye *et al.*[1] but could reflect physiological cleavage events, such as the excision of small regulatory RNAs harbored within introns or recursive splicing events. Curly red lines, introns; blue, exons; ss, splice site; A, branchpoint (bulged adenosine); gray balls, Pol II; pink balloons, RNPs.

The author is at the Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany.
e-mail: neugebau@mpi-cbg.de

process that does not require Pol II. Indeed, multiple protein complexes are known to associate with the nascent mRNA. For example, assembly of the nascent RNP probably begins with the cap-binding complex, which binds the 5′ end of the RNA shortly after transcription initiation[7]. Moreover, early studies led to the concept that RNA is bound by any number of RNA-binding proteins, such as heterogenous nuclear RNP proteins, as soon as it is made[8,9]. Strikingly, members of the SR protein family of splicing factors can hold exons together *in vitro*, with introns looping out[10], raising the possibility that these and/or other RNA-binding proteins associate with nascent RNA to hold distinct regions in close proximity during transcription, without the direct involvement of Pol II. This issue will need to be resolved in the future by driving the splicing constructs used in the Dye *et al*. study with RNA polymerases other than Pol II.

Although the concept that splicing is cotranscriptional is becoming dogma, it is important to remember that the same studies showing cotranscriptional splicing also document the likelihood that much of splicing occurs post-transcriptionally. For example, the levels of cotranscriptional intron removal decline for downstream introns, suggesting that RNAs that are unspliced at the time of transcription termination are spliced after nascent RNP release from chromatin[11]. It is currently unknown whether or for how long Pol II remains associated with RNPs released from transcription units. From this point of view, it might make more sense if the holding process within the nascent RNP did not rely on Pol II. Alternatively, Pol II could remain associated with nascent RNPs until splicing is complete and might be specifically released from RNPs, perhaps in conjunction with the Pol II recycling process.

The results of Dye *et al.*[1] now lead us to imagine that, analogously to a phone queue, exons will be spliced together in the order in which they are synthesized. Immediately, we recoil at the draconian order imposed by such a splicing queue, because it is at odds with the phenomenon of alternative splicing, which is frequent in metazoans and often involves exon skipping[2]. A logical explanation is that, during alternative splicing, an exon may not be defined as such and may therefore be bumped from the line, allowing the next synthesized exon to replace it in the queue. In this sense, the excluded exon becomes part of the intron. However, studies showing a kinetic relationship between transcription and alternative splicing dictate that alternative exons can be rescued and included either by slower mechanisms of exon definition or by slowing the rate of transcription elongation, or both[12–14]. Perhaps intron sequences are not too far away for alternative exons to be added back into the queue.

During cotranscriptional splicing, one can imagine that the 'first' exon becomes progressively longer as subsequent exons are spliced to it (**Fig. 1**). A question for future investigation will be whether distinct exons are held independently or whether only the first, continuously growing exon is held in place. Either way, one can speculate that the holding process creates an exon-rich zone or platform within the nascent RNP that may influence later splicing events. For example, spliced exons—or even accumulated unspliced exons—may create new binding sites for splicing regulators that may interact with downstream regions of the transcript. This might help explain observations of so-called 'polar effects,' in which an upstream alternative exon can influence the inclusion of a downstream alternative exon within the same transcript[15]. In this scenario, alternative exon definition might take place in the context of the splicing queue, with the included alternative upstream exon held and waiting to communicate with the distant one. Although the mechanisms underlying these phenomena and the ramifications for alternative splicing now need to be explored, one thing seems clear: you will eventually get your message through, if you hold the line.

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Telomeric proteins: clearing the way for the replication fork

Jan Karlseder

Protein complexes at telomeres have been assumed to present an obstacle to the passing replication fork. The observation that the *Schizosaccharomyces pombe* telomere-binding protein Taz1 is required for replication suggests otherwise.

The 'end-replication problem,' defined as the inability of the conventional DNA-replication machinery to fully replicate linear DNA molecules[1,2], as well as the discovery of telomerase, the reverse transcriptase that specifically elongates the 3′ chromosome terminus[3], has led many scientists to focus on telomerase. As a result, the question of how the moving replication fork passes through the G-rich and protein-rich sequences at chromosome ends has been neglected. This has now changed with a recent report in *Nature*, in which Miller *et al.*[4] have demonstrated that the *S. pombe* telomeric protein Taz1 is required for efficient replication of yeast telomeres. This observation rebukes the hypothesis that protein complexes covering the G-rich repeats inhibit DNA replication *in vivo*. On the contrary, loss of Taz1 brought replication to a halt at telomeric sequences not only at chromosome ends, but also when they were placed in the middle of chromosomes. Stalling of replication forks is independent of polarity, as telomeric repeats represented an obstacle to the moving fork no matter whether

The author is at the Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, California 92037, USA.
e-mail: karlseder@salk.edu