Cotranscriptional splicing regulation: it's not just about speed

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Recent data suggest that the C-terminal domain of RNA polymerase II can repress exon inclusion via a mechanism not explained by the prevailing models for cotranscriptional splicing regulation.

Alternative splicing, the differential inclusion or skipping of individual exons from a final messenger RNA, occurs in upwards of 75% of human genes and may be a primary mechanism for generating protein diversity and regulating protein expression. For an exon to be included in a final mRNA, the splicing machinery, or spliceosome, must be able to bind the exon and assemble it into a proper catalytic conformation. However, accurate exon recognition in mammals is complicated by the fact that each gene contains numerous small (<300 bp) exons surrounded by large introns (~3 kb average), and also that spliceosome binding sequences at exon-intron boundaries are highly degenerate. Therefore, to a first approximation, the pattern of exon joining that prevails for any given transcript results from the net effect of positive and negative factors that, respectively, enhance and hinder interaction of each exon with the spliceosome[1]. Although early work on alternative splicing focused solely on the effects of regulatory proteins that bind directly to enhancer or silencer sequences within the mRNA, more recent work has highlighted the importance of transcription in directing interactions between the spliceosome and individual exons within a precursor mRNA[2–5]. Thus far, two models have been suggested for the way transcription influences splicing: a kinetic model and a recruitment model, both detailed below. On page 973 of this issue, de la Mata and Kornblihtt[6] provide new insight into how transcription can influence alternative splicing apart from any kinetic effects of elongation.

The mechanism that has gained the most experimental support thus far for how transcription can influence alternative splicing patterns is described by a kinetic model, also known colloquially as the 'first come, first served' model, which has recently been described in detail by others[2]. Briefly, this model is based on the fact that, given the length of a typical mammalian intron, the time lag between the transcription of one exon and the transcription of the next exon is often long enough that the first exon can be bound by the spliceosome before the next exon is present (see **Fig. 1**). This time lag potentially allows a 'weak' exon (that is, an exon with suboptimal splice sites or silencer elements) to be recognized by the spliceosome without having to compete with a subsequent 'strong' exon. It follows, then, that slowing transcriptional elongation favors the recognition and inclusion of weak exons, whereas increasing the rate of transcription favors exon skipping. Indeed, exactly such data have been obtained now by several groups[7–9].

An alternative to the kinetic model is the recruitment model. Initially, the recruitment model gained strong support from the finding that numerous splicing factors can interact with components of the RNA polymerase II (Pol II) transcription complex. In particular, the C-terminal domain (CTD) of Pol II has been shown to interact directly with the spliceosomal proteins Prp40 and PSF, as well as a family of splicing-related proteins called SCAFs[10–12]. The CTD is a highly dynamic region of Pol II consisting of 52 repeats of a heptameric amino acid sequence that is differentially phosphorylated during the transcription cycle and can assume multiple conformations to allow interaction with a plethora of proteins[13]. In its simplest form, the recruitment model states that binding of splicing factors to the CTD increases their local concentration in the proximity of the nascent transcript, thereby promoting otherwise weak interactions between the splicing factors and the precursor mRNA (**Fig. 2a**). Though this model is no doubt attractive, it has been less well supported by experimental data. In particular, it has been suggested that the overall concentration of splicing factors in the nucleus is high enough that an increase in local concentration is not necessary to promote protein-RNA interactions[14]. Therefore, although the recruitment model remains a possibility, the weight of recent evidence has favored the kinetic model as the primary mode of transcription-dependent regulation of alternative splicing. Interestingly, de la Mata and Kornblihtt now put forth new data that at least argues in favor of the recruitment model and perhaps even suggests a more novel mechanism for transcription-dependent splicing regulation[6].

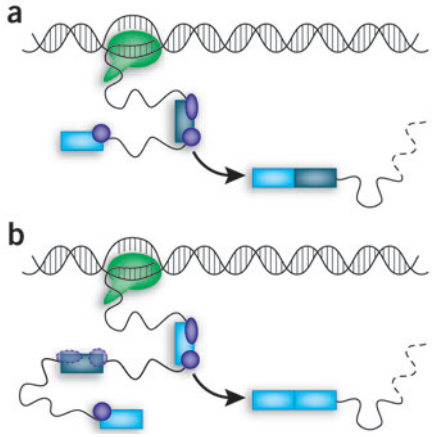

Figure 1 Kinetic model for transcription-dependent alternative splicing. (**a**) Binding of spliceosomal components to a weak exon is promoted when Pol II elongation is slowed. (**b**) Faster elongation results in skipping of the weak exon. Green, Pol II and the CTD; purple ovals, components of the spliceosome; dark blue box, weak exon; light blue boxes, flanking strong exons.

For several years, the Kornblihtt laboratory has studied the coupling of transcription and splicing. In previous studies, this group has reported both promoter-dependent and elongation-dependent effects on the inclusion of the alternative EDI exon of the fibronectin gene[7,15]. Given these links between transcription and splicing, de la Mata and Kornblihtt set out to investigate the role of the CTD in determining EDI inclusion. They used a clever inducible system that allows them to specifically drive expression of an EDI splicing reporter minigene with α-amanitin–resistant versions of Pol II that they introduce into the cell. Using this system, the authors show that mRNAs transcribed with a version of Pol II lacking the CTD (ΔCTD) have a much higher percentage of EDI inclusion than mRNAs transcribed with a CTD-containing Pol II. This demonstrates that the CTD is necessary for normal EDI silencing[6]. The ability of the CTD to repress EDI inclusion does not require a specific CTD sequence

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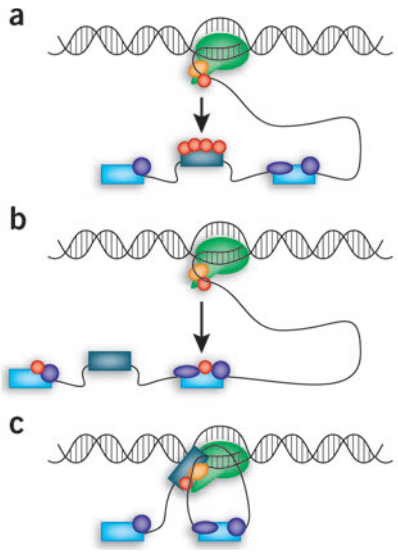

Figure 2 Alternative models for transcription-dependent alternative splicing (diagrammed as in **Fig. 1**). Shown are possible mechanisms for silencing of EDI when SRp20 (red) is recruited to the CTD through indirect interactions with additional transcription complex components such as mediator (orange). (**a**) Recruitment model showing SRp20 recruited by the CTD being transferred to the weak EDI exon and repressing spliceosome binding of the exon. (**b**) Alternative recruitment model, in which SRp20 is recruited to the flanking exons to promote exon recognition. In this model, EDI is skipped because SRp20 does not bind. (**c**) Sequestration model, in which the EDI exon is bound up in a large SRp20–CTD–Pol II complex, making it inaccessible to the splicing machinery.

but rather correlates with the number of heptad repeats. Surprisingly, the authors did not observe a general inhibition of splicing with the ΔCTD Pol II, as has been reported by others[16]. However, because they show only that overall splicing of the minigene with ΔCTD Pol II is equivalent to splicing in the presence of wild-type polymerase, this result may reflect transcript-specific differences in sensitivity to decreased levels of CTD-containing Pol II.

Given previous data and the models described above, CTD-dependent silencing could easily be explained in one of two ways. Either the CTD could enhance elongation, thus speeding Pol II through the weak EDI exon to the downstream exon and promoting EDI skipping, or the CTD could help carry promoter-specific repressing proteins to silencer elements within the EDI exon. However, strikingly, de la Mata and Kornblihtt demonstrate that the CTD-dependent silencing of EDI is neither promoter dependent nor influenced by elongation rate. Instead, the CTD-dependent silencing of EDI seems to be mediated by SRp20, a member of the SR protein family of splicing factors. SRp20 was previously implicated by the Kornblihtt group in mediating EDI skipping[15]. Importantly, deletion of the CTD or knockdown of SRp20 each promote EDI inclusion to an equivalent extent, and removal of both does not further increase EDI inclusion, demonstrating that CTD and SRp20 function by redundant mechanisms. In contrast, the ability of the SR protein SF2/ASF to enhance EDI inclusion is unaffected by the presence or absence of the CTD.

The data presented here by de la Mata and Kornblihtt[6] clearly show that Pol II, through its CTD, can influence EDI inclusion by a mechanism that cannot be explained in terms of elongation. But what, really, do we know about how the CTD and SRp20 function, and is this truly evidence for the recruitment model in its simplest terms (**Fig. 2a**), or might these data suggest a more novel mechanism? There are no reports as to how SRp20 functions to repress EDI once bound to this exon (if, indeed, it does bind), and de la Mata and Kornblihtt[6] state that they find no evidence of a direct interaction between the CTD and SRp20, although SRp20 has been identified as part of the Pol II mediator complex[17]. The authors suggest two possible variations of the recruitment model for the function of SRp20 and the CTD: SRp20 bound indirectly to the CTD (i) specifically inhibits EDI or (ii) specifically promotes spliceosome recognition of the flanking exons (**Fig. 2a**,**b**, respectively). However, neither model truly explains the requirement for the CTD or how the specificity is achieved. An alternative model worth considering is shown in **Figure 2c**. In this model, SRp20 bound to EDI would recruit the exon to the Pol II–CTD–mediator complex, thereby effectively sequestering it away from the spliceosome. This model is similar to results in recent studies showing that sequestering of an exon in a sterically hindered or unproductive complex can result in exon skipping[18,19]. Moreover, it is the logical extension of a model suggested previously[20] to explain CTD-dependent enhancement of exon pairing, in which transient association of an exon with the Pol II complex could help hold it in the vicinity of the next exon. Clearly, much more work needs to be done to rule any of these models in or out. But given the complexity of cotranscriptional splicing and the potential for new insight into mechanisms of alternative splicing, further investigation of the system described by de la Mata and Kornblihtt should continue to provide much information regarding the interplay of transcription and splicing.

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