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Pre-mRNA Splicing in the Nuclear Landscape

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Abstract

Eukaryotic gene expression requires the cumulative activity of multiple molecular machines to synthesize and process newly transcribed pre-messenger RNA. Introns, the noncoding regions in pre-mRNA, must be removed by the spliceosome, which assembles on the pre-mRNA as it is transcribed by RNA polymerase II (Pol II). The assembly and activity of the spliceosome can be modulated by features including the speed of transcription elongation, chromatin, post-translational modifications of Pol II and histone tails, and other RNA processing events like 5′-end capping. Here, we review recent work that has revealed cooperation and coordination among co-transcriptional processing events and speculate on new avenues of research. We anticipate new mechanistic insights capable of unraveling the relative contribution of coupled processing to gene expression.

Eukaryotic pre-messenger RNA (pre-mRNA) is processed in the nuclear milieu by multiple molecular machines working in concert to affect gene expression. Pre-mRNA processing starts concurrently with transcription elongation (i.e., co-transcriptionally) and likely proceeds until the mRNA is packaged for export to the cytoplasm (Fig. 1; Beyer and Osheim 1988; Baurén and Wieslander 1994). A major component of pre-mRNA processing is RNA splicing, which excises noncoding, intervening regions (introns) from a transcript to generate mRNA. Introns can be plentiful in eukaryotic genes, and the selective removal of introns can significantly impact gene expression by altering transcript stability, coding potential, or localization. Some of the first evidence for co-transcriptional processing was the transcription-dependent recruitment of splicing factors to chromatin (Sass and Pederson 1984). In the past 10 years, global analyses have revealed that co-transcriptional removal of introns is conserved from yeast to humans (Carrillo Oesterreich et al. 2010; Ameur et al. 2011; Khodor et al. 2011, 2012; Schmidt et al. 2011; Girard et al. 2012; Tilgner et al. 2012; Windhager et al. 2012; Nojima et al. 2015; Pai et al. 2017).

An increasingly prominent theme in molecular biology is that the complexes responsible for synthesizing and modifying mRNA cross-regulate to fine-tune gene outputs. For instance, the presence of an intron in transgenes is positively correlated with transcriptional activity (Brinster et al. 1988), intron–exon boundaries are associated with active chromatin marks (Bieberstein et al. 2012), and splicing factors stimulate in vitro transcription reactions (Fong

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and Zhou 2001). This evidence suggests coordination between transcription and splicing. It is also well known that RNA polymerase II (Pol II) speed influences alternative splicing and intron retention in all examined species (Schor et al. 2009, 2013; Aslanzadeh et al. 2018; Saldi et al. 2018; Godoy Herz et al. 2019). Moreover, transcripts with multiple introns tend to have either all the introns removed or all the introns retained in *Schizosaccharomyces pombe*, suggesting coordination or cross-regulation between individual splicing events (Herzel et al. 2018). This may also be true in insect and mammalian cells, because coordination among adjacent introns has been well documented (Tilgner et al. 2015, 2018; Kim et al. 2017; Pai et al. 2017; Drexler et al. 2020). Furthermore, pre-mRNAs that are spliced are also more efficiently cleaved for polyadenylation, suggesting a relationship between the two machineries (Davidson and West 2013; Herzel et al. 2018). At the other end of the gene, we know that capping of the pre-mRNA 5′ end occurs in a rapid and coordinated fashion with transcription initiation (Hagler and Shuman 1992; Rasmussen and Lis 1993) and that promoter identity plays a role in alternative splicing and even RNA half-life (Cramer et al. 1997; Trcek et al. 2011; Fiszbein et al. 2019).

Over the years, major advances in understanding coupled processing events have emerged from new technologies. Here, we review recent progress on understanding coupled and coordinated pre-mRNA splicing focusing on the yeast *Saccharomyces cerevisiae*. Budding yeast is a powerful system for uncovering the mechanisms of splicing and transcription coordination, because although only 300 genes have introns, these encoded intron-containing mRNAs account for 25%–30% of the transcripts in yeast cells (Ares et al. 1999; Lopez and Seraphin 1999). Indeed, the fitness of yeast depends on having introns as the physical presence of introns in genes can promote survival in starvation conditions (Parenteau et al. 2019). The yeast spliceosome has also served as a useful model for understanding the effect of splicing factor mutations identified in human diseases including various hematopoietic malignancies and retinitis pigmentosa (Tang et al. 2016; Carrocci et al. 2017; Ruzickova and Stanek 2017).

THE MECHANISM OF PRE-mRNA SPLICING

Pre-mRNA splicing is catalyzed by the spliceosome, a single-turnover enzyme composed of the U1, U2, U4, U5, and U6 small nuclear ribonucleoproteins (snRNP) and a number of protein-only factors (Lerner et al. 1980). Early work identified several splicing factors as temperature-sensitive mutants in yeast (Hartwell 1967; Hartwell et al. 1970). The spliceosome defines the intron through recognition of conserved sequences located in the intron: the 5′ splice site (5′ss), the branchpoint sequence (bps), and the 3′ splice site (3′ss). Splice site recognition is aided by the activity of non-snRNP factors, such as Mud2 and branchpoint binding protein (BBP), which promote pairing between the 5′ss and 3′ss (Abovich and Rosbash 1997; Lacadie et al. 2006). The intronic sequences are obligate components of the splicing reaction and form part of the spliceosome active site during catalysis. Splicing occurs in two sequential trans-esterification reactions (Fig. 2; Moore and Sharp 1993). The catalytic steps of splicing are ATP-independent, but ATP is required for the activity of a number of ATPases that structurally and compositionally remodel the spliceosome during assembly and catalysis (Liu and Cheng 2015). In the first reaction, the branchpoint adenosine acts as the nucleophile to cleave the phosphodiester backbone at the 5

exon–intron boundary and generate a free 5′ exon and lariat intron–3′ exon intermediate. During the second step, the 3′OH of the 5′ exon acts as the nucleophile to excise the lariat intron and ligate the exons together.

The spliceosome can use a variety of splice sites to varying degrees of efficiency and the conservation of splice sites differs greatly among organisms (Will and Luhrmann 2011; Qin et al. 2016). Splice sites in the yeast *S. cerevisiae* are very well conserved and rarely diverge from the consensus sequence, whereas metazoan splice sites are more degenerate. This is in part due to the presence of splicing regulatory proteins in metazoans that recognize splicing regulatory elements and influence splice site usage (Zhong et al. 2009). In yeast, splicing regulation has been attributed to Npl3 and Nam8. Npl3 is an SR-like protein, which is required for proper spliceosome assembly in the co-transcriptional context, and *NPL3* mutations lead to intron retention (Kress et al. 2008). Interestingly, splice site usage can be further modified by the surrounding sequence context. In the context of a minigene reporter system, Wong et al. (2018) showed that the spliceosome can accommodate different 5′ss sequences depending on the origin of the intron. In yeast, Nam8 is a poly(U) binding protein that binds near the 5′ss and enhances 5′ss recognition by the U1 snRNP in a manner analogous to TIA-1 in humans (Puig et al. 1999; Förch et al. 2000; Spingola and Ares 2000; Qiu et al. 2011).

The steps of spliceosome assembly have been extensively characterized using in vitro biochemistry and genetics (Fig. 2) (Will and Luhrmann 2011). More recently, the molecular architecture of many intermediate complexes along the splicing reaction have been revealed using cryo-EM (reviewed extensively in Fica and Nagai 2017; Shi 2017; Wilkinson et al. 2019). Initially, the 5′ end of the U1 snRNA base pairs with the 5′ss found at the 5′ boundary of the intron to form the spliceosome E complex. Association of the U2 snRNP with the bps/3′ss region converts the E complex to the A complex. A complex is converted to the pre-B complex upon addition of the U4/U6.U5 tri-snRNP and subsequently to the B complex upon exchange of base pairing at the 5′ss from the U1 snRNA to the U6 snRNA and release of the U1 snRNP. Unwinding of U4/U6 base pairing and release of the U4 snRNA permits the formation of the U2/U6 catalytic active site and marks formation of the B^{act} complex. The spliceosome is then heavily remodeled to bring together the U2/U6 duplex and the U2/bps duplex (B* complex). Splicing factors then activate the spliceosome to promote the catalytic steps in the C and C* complexes to ligate the exons and form the postcatalytic P complex. The P complex spliceosome is then released from the mRNA and disassembled. Splicing is energetically costly and the spliceosome must assemble anew from its constituent components for every intron. This means that in mammalian cells, where genes contain on average eight introns, eight spliceosomes must assemble and disassemble for every pre-mRNA synthesized. In yeast, the majority of genes lack introns (Spingola et al. 1999); yet, the fact that 35% of transcripts must be spliced generates a strong demand for spliceosomal components. Reduced demand for splicing leads to a “hungry spliceosome,” which can act on cryptic splice sites in RNA that are not normally recognized by the spliceosome (Munding et al. 2013; Talkish et al. 2019).

CO-TRANSCRIPTIONAL ASSEMBLY OF THE SPLICEOSOME

Studies from a number of laboratories and in many species have shown that pre-mRNA splicing occurs concurrently with transcription elongation by Pol II (Carrillo Oesterreich et al. 2010; Ameur et al. 2011; Khodor et al. 2011, 2012; Schmidt et al. 2011; Girard et al. 2012; Tilgner et al. 2012; Windhager et al. 2012; Nojima et al. 2015; Pai et al. 2017). If splicing occurs co-transcriptionally, then so must spliceosome assembly. This scenario raises the possibility that components of the spliceosome would associate with the nascent RNA potentially as soon as the corresponding RNA element is synthesized (e.g., the U1 snRNP would bind the RNA as soon as Pol II has transcribed the 5′ss). Additional possibilities include the potential for Pol II itself and/or components of chromatin to help recruit spliceosomal components. The work of our laboratory and others established that splicing factors associate with gene bodies in vivo in a manner that mirrors the stepwise assembly pathway defined in vitro (Kotovic et al. 2003; Gornemann et al. 2005; Tardiff et al. 2006; Hoskins et al. 2011). Because splicing occurs co-transcriptionally, splicing factors are adjacent to the DNA axis and can cross-link to the underlying chromatin (Fig. 3A). These "splicing factor ChIP" experiments showed that U1 snRNP signal peaks in the region downstream from the 5′ exon–intron boundary and decays downstream from the intron–3′exon boundary (Fig. 3B) (Kotovic et al. 2003; Tardiff et al. 2006). Furthermore, U2 and U5 snRNPs peaked in the downstream exon, consistent with the addition of U2 and U5 subsequent to both U1 binding and synthesis of the 3′ss. ChIP data were later extended by high resolution cross-linking and immunoprecipitation (CLIP) experiments that identified the binding sites of many splicing factors on the mRNA substrate (Fig. 3C). For example, it was unclear how Mud2—similar to metazoan U2AF that binds the polypyrimidine tract at the 3′ss —might participate in gene expression in yeast, which generally lack a polypyrimidine tract. Mud2 CLIP shows that this protein binds along the entire length of the intron, displaying a different role in mRNA processing than suspected from protein homology (Baejen et al. 2014).

Broadly speaking, the ChIP and CLIP profiles in budding yeast were consistent with spliceosome assembly during transcription, but the degree to which splicing completed co-transcriptionally was contentious, with some studies concluding that exon ligation could not complete before cleavage at the poly(A) site (Tardiff et al. 2006). However, our recent work has suggested that splicing can occur rapidly relative to transcription of the 3′ss and is often upstream of previously identified ChIP peaks (Tardiff et al. 2006; Oesterreich et al. 2016; Wallace and Beggs 2017). This discrepancy is likely in part due to the low resolution of ChIP data compared to more recent custom sequencing strategies like single-molecule intron tracking and long-read sequencing, which identify the position of Pol II with single-nucleotide resolution while simultaneously determining the transcript splicing status. Spliced products can be identified while Pol II is still in close proximity to the 3′ss (Fig. 3D), suggesting Pol II and the spliceosome are physically close and corroborating earlier measurements of efficient co-transcriptional splicing with high-density tiling arrays (Carrillo Oesterreich et al. 2010). Taken together, these findings suggest that although the chemistry of RNA splicing may occur quickly, spliceosomal components may be retained in downstream gene regions because of the slow release of the post-catalytic spliceosome. This

is an interesting observation because both chemical steps of splicing have been shown to be reversible in vitro (Tseng and Cheng 2008) and might suggest that an analogous complex could exist in vivo.

Simultaneous transcription and splicing afford the opportunity for cross-regulation and demand coupling for accurate gene expression. Splice site sequences are short and consensus sequences alone are insufficient to identify introns because of the presence of near-cognates in the genome. How can co-transcriptional processing aid the spliceosome in selecting the correct sites? Co-transcriptional splicing reduces the number of potential splice sites to only the subset of those in the transcript that have been transcribed at the time of spliceosome assembly. This idea led to the “first come, first served” model of splice usage which states that the splice sites that are transcribed first are used by the spliceosome (Kuhne et al. 1983). The Kornblihtt laboratory subsequently showed that “first come, first served” could refer to splicing commitment and not necessarily the completion of intron removal in the order of synthesis (de la Mata et al. 2010). Concordantly, changes in the rate of Pol II elongation can lead to changes in the outcomes of splicing even in budding yeast (Howe et al. 2003; Braberg et al. 2013; Oesterreich et al. 2016; Aslanzadeh et al. 2018). Interestingly, both faster and slower elongation leads to changes, suggesting that the transcription and splicing machinery are finely tuned to work concurrently.

Pol II elongation along gene bodies in human cells is nonuniform, with elongation rates slower over exons than introns (Veloso et al. 2014). In budding and fission yeasts, high-resolution elongation rate measurements are difficult to make and have not been accomplished; instead, a single study established an average elongation rate in budding yeast of 1.5 kb/min (Mason and Struhl 2005). Reduction in Pol II elongation (i.e., transcriptional pausing) has been proposed as an important determinant of splicing, particularly when excising introns with nonconsensus splice sites (Aslanzadeh et al. 2018). Changes in the overall rate of transcription has broad effects on splicing efficiency, indicating tuning between the rates of two processes (Oesterreich et al. 2016). Indeed, reduction in elongation rate either through mutation of Pol II or through drug treatment can rescue middle exon inclusion in multi-intron yeast genes (Howe et al. 2003). Terminal exon pausing has been identified in short endogenous genes that are spliced efficiently co-transcriptionally but the molecular features that contribute to pausing remain unclear (Carrillo Oesterreich et al. 2010). Understanding the functional consequences of pausing in terminal exons is difficult, because we currently lack the tools to manipulate pausing per se; changing the overall rate of transcription is not the same as modulating elongation at particular gene locations. Theoretically, pausing should play a role in the extent of transcripts that are spliced co-transcriptionally, but the rapid appearance of spliced products relative to transcription progress suggests that pausing may not be absolutely required for splicing. Dissecting the contribution of transcriptional pausing to efficient co-transcriptional processing will require the development of new tools to manipulate pausing on genes in their native context.

Perturbation of pre-mRNA splicing also feeds back to modify transcription. Splicing has been proposed to be a checkpoint for Pol II pausing because splicing inhibition can lead to an increase in Pol II ChIP signal over introns, further supporting communication between the two machines (Alexander et al. 2010; Chathoth et al. 2014). More recently, splicing

factors, such as Mud2 and Npl3, have also been suggested to act in transcription elongation (Dermody et al. 2008; Minocha et al. 2018). Nevertheless, the molecular mechanism by which Mud2 or Npl3 contributes to efficient elongation remains unknown.

POL II MODIFICATIONS AND THE SPLICEOSOME

The major proposed mechanism of communication between elongating Pol II and complexes that modify the nascent RNA is the Pol II carboxy-terminal domain (CTD) (Custodio and Carmo-Fonseca 2016; Harlen and Churchman 2017; Wallace and Beggs 2017). The CTD is highly conserved among eukaryotes and contains a species-specific number of heptad repeats of the sequence Tyr1–Ser2–Pro3–Thr4–Ser5–Pro6–Ser7 (26 repeats in *S. cerevisiae* and 52 in humans). Although these repeats may act as a platform to assist in the recruitment of splicing factors to the nascent RNA, the CTD alone does not specify recruitment; for example, snRNPs are not recruited to intronless genes in yeast (Kotovic et al. 2003; Gornemann et al. 2005). Further, a CTD domain is not strictly required for splicing. Splicing occurs in the absence of transcription in vitro, and even in the context of an elongating polymerase there is no significant change in the efficiency of splicing between complexes that do and do not have a CTD (Lin et al. 1985; Natalizio et al. 2009). Eukaryotic genes including the *S. pombe* U6 gene that are transcribed by RNA polymerase III, which lacks a CTD, can be efficiently spliced by the spliceosome (Tani and Ohshima 1989). Nevertheless, phosphorylation of Pol II stimulates pre-mRNA splicing (Hirose et al. 1999), suggesting that splicing factor recruitment is modulated by kinases and phosphatases that dynamically post-translationally modify the CTD as Pol II transcribes along the body of the gene. Importantly, mNET-seq data from human cells has shown that Ser5P antibodies precipitate intermediates of the splicing reaction including free 5′ exons and lariat intron–3′ exon intermediates (Mayer et al. 2015; Nojima et al. 2015, 2018). This indicates that nascent RNAs that are undergoing splicing are attached to Pol II molecules with this modification. Indeed, antibodies targeting the Ser5P phospho-epitope immunoprecipitate splicing factors alongside the polymerase (Harlen et al. 2016). Additionally, pre-mRNAs transcribed by Pol II are more efficiently processed in vitro than pre-mRNAs transcribed by another polymerase lacking a CTD (Das et al. 2006). Together, these data suggest that the modified CTD may interact with the spliceosome but that other features may also significantly contribute to splicing outcomes.

CONNECTIONS TO THE mRNA CAP

The 5′ end of mRNA is capped with an inverted, methylated guanosine nucleotide that plays a major role in the life of the transcript, including mRNA splicing, 3′-end formation, export, and overall stability (Ramanathan et al. 2016). The cap is one of the first modifications installed on eukaryotic mRNA and the effects of the cap are mediated through interaction with the cap-binding complex (CBC). The CBC binds to the nascent RNA early during transcription and may help promote escape from the promoter (Lidschreiber et al. 2013). The CBC has been shown to be important, but not essential, for efficient pre-mRNA splicing in both mammalian and yeast systems (Fresco and Buratowski 1996; Schwer and Shuman 1996). Defects in mRNA capping lead to the accumulation of unspliced precursors. The CBC helps to promote E complex formation by interacting directly with U1 to recruit the

snRNP and stabilize it at the 5′ss to promote spliceosome assembly (Fig. 4A,B; Lewis et al. 1996; Gornemann et al. 2005; Larson and Hoskins 2017; Li et al. 2019). Furthermore, the CBC has been proposed to play a role in the stable recruitment of the tri-snRNP (Pabis et al. 2013). Therefore, interactions with the 5′ cap of the mRNA have been proposed to be a major determinant of how the transcript will be processed. Interestingly, Ser5 phosphorylation of the Pol II CTD has also been shown to be important for RNA capping (Fabrega et al. 2003). The lethality associated with Ser5A Pol II CTD mutant in *S. pombe* can be bypassed by fusion of the mammalian capping enzyme to Pol II (Schwer et al. 2012). Therefore, whether the Ser5 phosphomark is directly required for splicing or whether it contributes indirectly by promoting efficient capping remains unclear.

CHROMATIN AND SPLICING

The co-transcriptional nature of splicing places the spliceosome in proximity to chromatin—the array of nucleosomes made up of DNA wrapped around eight histones. Some early indication of the impact of chromatin on the regulation of splicing came from work in which the integration of the adenovirus genome at different genomic locations resulted in altered splicing outcomes (Adami and Babiss 1991). In metazoans, nucleosomes are found more frequently at exons than introns, and it is proposed that they act as landmarks of exons (Kogan and Trifonov 2005; Spies et al. 2009; Tilgner et al. 2009). Nucleosome positioning can act as a transcriptional barrier that might aid in the definition of exons and subsequent assembly of the spliceosome through transient polymerase pausing (Hodges et al. 2009). Independent of nucleosome position, gene architecture—namely, the arrangement of introns and exons in any given gene—has a profound effect on the distribution of histone post-translational modifications (PTMs), which likely impacts the splicing-dependent amplification of gene output. Specifically, H3K4me3 and other active marks are best aligned to the first 5′ss in genes, even better than alignment to transcription start sites; importantly, these peaks can be moved in the gene by changing its architecture (Bieberstein et al. 2012). This suggests that components of the splicing machinery enhance gene output in part through an uncharacterized interaction with histone modifying enzymes. Consistent with this idea, perturbations of histone deacetylases (HDACs) shift alternative splicing patterns globally (Hnilicova et al. 2011). Specific and physiologically relevant examples of histone PTM alterations that determine alternative splicing patterns include the changed chromatin landscape of the *NCAM* gene in stimulated or differentiating neurons (Schor et al. 2009, 2013). It is very difficult to disentangle the roles of chromatin, transcription rates, and splicing, which seem to have arrows pointing in all directions. This experimental obstacle may explain why relatively few specific examples of regulation, such as the targeting of an alternative splicing regulator to chromatin sites, have emerged (Sims et al. 2007; Luco and Misteli 2011; Kfir et al. 2015). Nevertheless, these seminal studies in metazoans show the complex roles the chromatin landscape can play in splicing regulation.

Although budding and fission yeasts essentially lack alternative splicing, chromatin plays a role in splicing efficiency. The recent high-throughput method epistatic mini array profile (E-MAP) has revealed links between splicing and chromatin (Braberg et al. 2013). For example, Npl3 was found to promote H2B monoubiquitination through interactions with Bre1 in budding yeast (Moehle et al. 2012), and the chromatin remodeler SWI/SNF was

linked to spliceosome activation in fission yeast (Patrick et al. 2015). Histone post-translational modifications have also been proposed to affect pre-mRNA splicing by directly modulating spliceosome assembly. The activity of the histone acetyltransferase Gcn5 promotes co-transcriptional U2 recruitment to the nascent RNA (Gunderson and Johnson 2009; Gunderson et al. 2011). More recently, genetic interactions between components of the U2 snRNP and the rare histone variant, H2A.Z, have been identified, pointing to a role in promoting splicing of introns with nonconsensus splice sites (Neves et al. 2017; Nissen et al. 2017). Furthermore, the histone methyltransferase Set2 and its corresponding H3K36 methylation mark has also been shown to play a role in the recruitment of splicing factors to the nascent transcript (Sorenson et al. 2016; Leung et al. 2019). Thus, although the molecular underpinnings of these linkages are unknown, the case for cross-regulation between chromatin and splicing is well-substantiated in the budding yeast system. Future work will be necessary to understand how this network of interactions translates into gene-specific splicing efficiencies.

OUTLOOK: PRE-mRNA SPLICING IN THE NUCLEAR LANDSCAPE

The nuclear landscape in metazoan cells is dominated by chromosomes and membraneless organelles, such as nucleoli, Cajal bodies, speckles, and others (Mao et al. 2011). Currently the field is engaged in exploring how these biomolecular condensates contribute to gene expression. It is becoming hard to imagine what nucleoplasm is, when even individual genes can be thought of as their own organelles with Pol II, RNA, and DNA- and RNA-binding proteins concentrated there (Herzel et al. 2017; Hnisz et al. 2017). Components of the transcription and splicing machinery are enriched in intrinsically disordered regions (IDRs) (Courchaine et al. 2016; Herzel et al. 2017), providing a logic for how small local condensates could form when nascent RNA is present. Indeed, SR proteins and the CTD of Pol II have some of the most extensive intrinsically disordered regions of all proteins. The concentration of SR proteins is high at transcription sites, and phosphorylation changes on the Pol II CTD during elongation could play a role in promoting splicing by recruiting snRNPs and other splicing factors, creating a high local concentration for the removal of multiple introns (Neugebauer and Roth 1997; Galganski et al. 2017; Herzel et al. 2017; Guo et al. 2019). At present, it is unclear how this understanding can be extended to budding and fission yeasts which often lack these long IDRs. Nevertheless, P-bodies were discovered in yeast (Sheth and Parker 2003), and yeast RNA has been shown to mediate condensation (Van Treeck et al. 2018). Yeast nucleoli are enormous, but speckles and Cajal bodies have not been observed. Therefore, importance of biomolecular condensation in yeast nuclei and its role in protein-coding gene expression is currently a frontier awaiting exploration.

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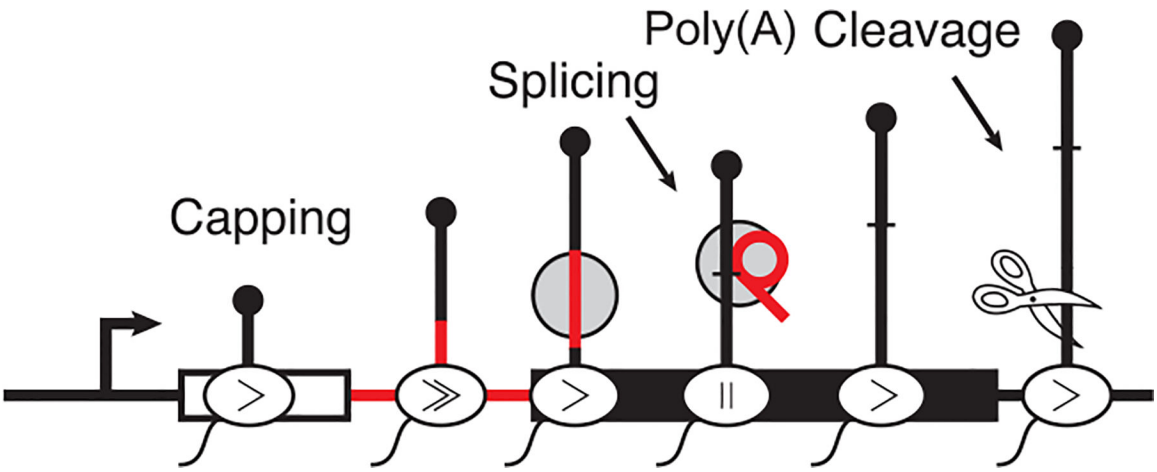

Figure 1.

Eukaryotic mRNA is processed concurrently with transcription to modify the transcript output. Processing can include 5′-end capping with 7-methylguanosine, splicing, and polyadenylation cleavage. The rate of elongation is also non-uniform along the gene body.

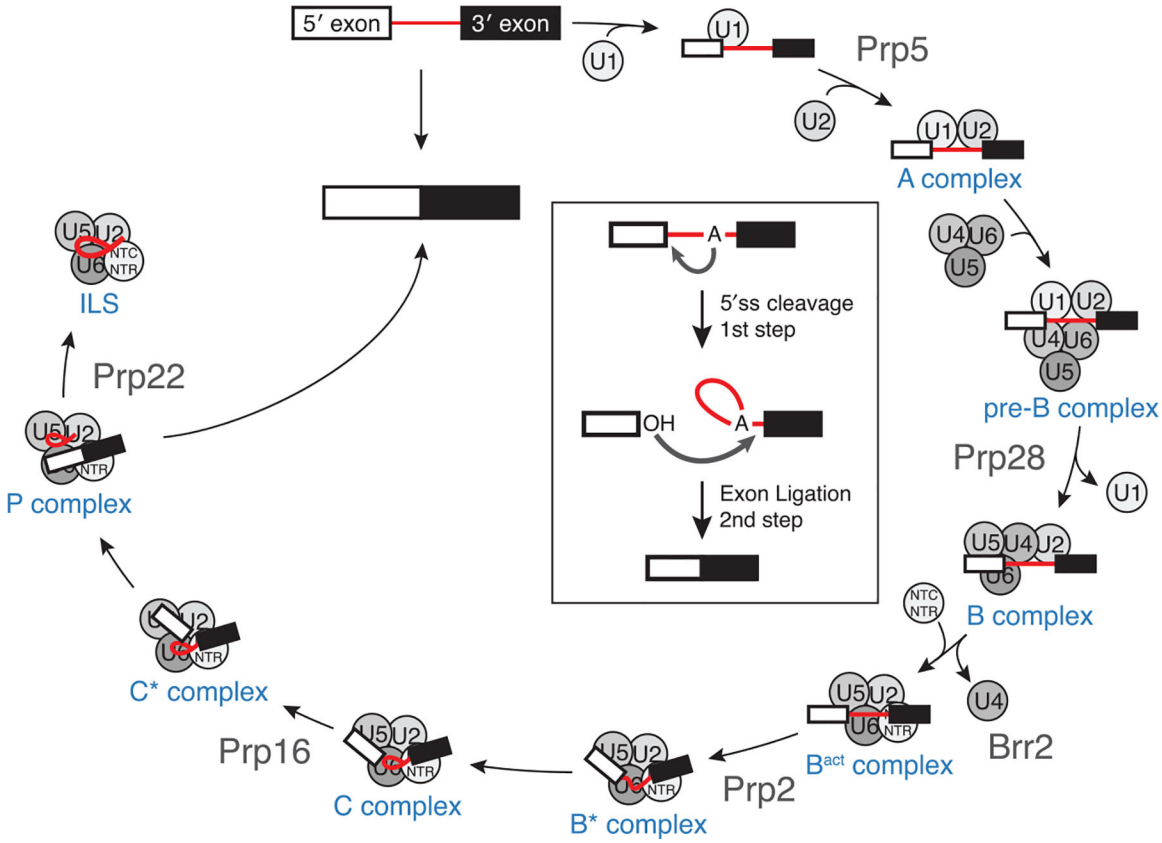

Figure 2.

Splicing occurs in two sequential chemical steps (*inset*) to excise the intron (red) and ligate the coding exons. The spliceosome assembles in an ordered manner from preassembled components responsible for identifying the splice sites, forming the active site, and catalysis. The spliceosome is then released and the components recycled for the next round of splicing. Splicing complex names are shown in blue, and helicases that help mediate major transitions in the splicing cycle are shown in gray.

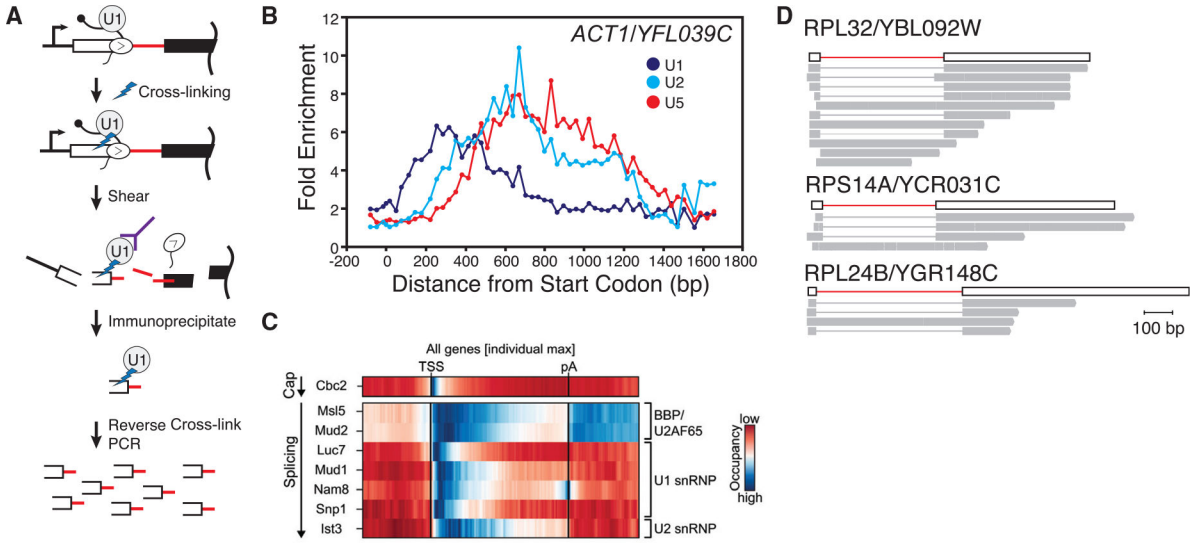

Figure 3.

Monitoring spliceosome assembly co-transcriptionally. (*A*) Schematic describing a splicing factor ChIP assay. Splicing factors (i.e., U1) are formaldehyde-cross-linked to the chromatin, and chromatin is subsequently fragmented. Amplification of fragments that coimmunoprecipitated infer the association of the targeted splicing factor along the gene body. (*B*) Representative spliceosome assembly profile for *ACT1/YFL039C*. U1 associates first and departs first. U2 addition is followed by U5, consistent with the ordered assembly model. (*C*) Splicing factor cross-linking immunoprecipitation identifying splicing factor association with the nascent RNA. (*D*) Representative long reads from yeast nascent RNA sequenced on the Pacific Biosciences RSII platform showing rapid splicing of the intron while Pol II is within ~200 nt of the 3′ss. Data reproduced from Oesterreich et al. (2016). (*B*, Reproduced, with permission from Tardiff et al. 2006; *C*, reproduced, with permission, from Baejen et al. 2014.)

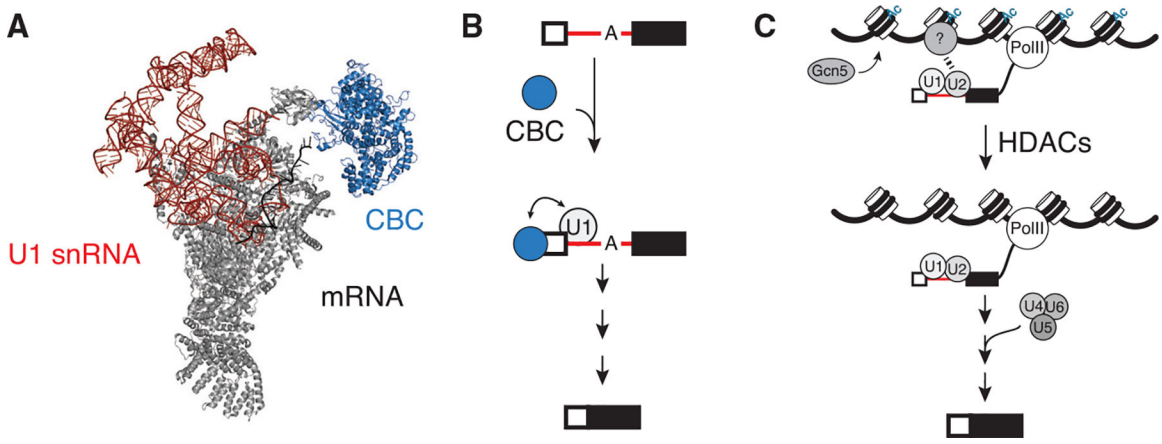

Figure 4.

Cap-binding complex aids in U1 recruitment. (*A*) The structure of the spliceosome E complex showed physical interactions with CBC and the U1 snRNP component Snp1 (U1–70K in humans; pdb 6N7P) (Li et al. 2019). (*B*) Capping of the nascent RNA recruits CBC, which in turn can nucleate spliceosome assembly and splicing. (*C*) Gcn5-dependent histone acetylation recruits an unknown factor to chromatin to modulate U2 recruitment. Deacetylation facilitates subsequent steps in splicing (Gunderson et al. 2011).