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Leading Edge
Review

Pre-mRNA Processing Reaches Back to Transcription and Ahead to Translation

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The pathway from gene activation in the nucleus to mRNA translation and decay at specific locations in the cytoplasm is both streamlined and highly interconnected. This review discusses how pre-mRNA processing, including 5′ cap addition, splicing, and polyadenylation, contributes to both the efficiency and fidelity of gene expression. The connections of pre-mRNA processing to upstream events in transcription and downstream events, including translation and mRNA decay, are elaborate, extensive, and remarkably interwoven.

In the 50 years since Crick's proposition of the central dogma of gene expression, there has been an explosion in our understanding of the steps involved in the flow of information from DNA to RNA to protein. Whereas many early studies focused on elucidating the machinery and mechanisms required for each individual step in the process, a conceptual transformation in the last decade came with the realization that gene activation in the nucleus and later events in the cytoplasm, such as translation and decay, are seamlessly integrated. In this review, we focus on how pre-mRNA processing regulates both transcription in the nucleus and the subsequent fate of an mRNA in the cytoplasm.

Nuclear Events

Before beginning this account, it is necessary to provide a brief reminder of the critical impact of structural studies on RNA polymerase II (Pol II) for our understanding of this gene expression "superhighway." Pol II has an overall globular structure with an enlarged central active site wherein the DNA template is forced apart as a single-stranded bubble (Cramer, 2004). Channels into this active site allow nucleotide access and RNA exit. Below the RNA exit channel lies a relatively unstructured protein domain, the carboxyl terminus of the largest Pol II subunit (Meinhart et al., 2005). This carboxyl terminal domain (CTD) has a simple heptad repeat structure, 26 repeats in *Saccharomyces cerevisiae* (henceforth referred to as yeast), 52 in mammals, and each repeat bears three serine residues that undergo reversible phosphorylation during the transcription cycle (Egloff and Murphy, 2008). Serine 5 phosphorylation (Ser5P) along the CTD repeats is a hallmark of early transcription elongation, whereas serine 2 phosphorylation (Ser2P) is associated with later-stage elongation (Komarnitsky et al., 2000). Reference will be made throughout our review to the key roles played by this structure.

Pol II: Poised for Action

A surprising discovery has emerged from genomic array analysis in both single-cell eukaryotes such as yeast and differentiated cells from multicellular organisms. This is that many gene promoters are permanently engaged with Pol II initiation complexes (Guenther et al., 2007; Muse et al., 2007) and may generate 5′ proximal abortive transcripts (Core and Lis, 2008; Kapranov et al., 2007). Indeed, recent studies demonstrate that short transcripts are generated in both the sense and antisense directions from these pre-engaged polymerases (summarized by Buratowski, 2008). The types of genes that possess pre-engaged Pol II are particularly genes regulated at specific development stages or inducible genes that need to rapidly respond to new extracellular situations. By having Pol II already plugged into the gene promoter, the complex process of uncovering the gene from repressive chromatin structure and recruiting the Pol II initiation complex from its component parts is bypassed. Exactly what the molecular trigger is that switches Pol II from abortive initiation into functional elongation mode is still largely unknown (Figure 1). However, it has become evident that pre-mRNA processing plays a critical role (Manley, 2002; Orphanides and Reinberg, 2002; Sims et al., 2007).

The first RNA processing event to occur on the nascent transcript is 5′ end capping. Three enzymatic activities, a triphosphatase, a guanyl transferase, and a methyl transferase, all act to convert the pppA 5′ terminus of the primary transcript to 7meGpppA (Shuman, 2001). The first two activities are present on a single polypeptide in mammals but reside on separate proteins, Ceg1 and Cet1, in yeast. All eukaryotes possess a separate methyltransferase. Ceg1 and Cet1 are recruited to the Pol II initiation complex once the CTD has become activated by Ser5P formation through the action of TFIIH-associated cyclin dependent kinase, Cdk7 (also known as Kin28 in *S. cerevisiae*; Schroeder et al., 2000). Ceg1 directly associates with CTD Ser5P, allowing it to act on nascent transcripts as soon as they emerge from the elongating Pol II. Capping may well be a key component of the switch that pushes Pol II from abortive early elongation into fully processive elongation across the body of the gene. Other components of this switch involve both negative and positive elongation factors that may themselves be regulated by phosphorylation dictated by cell signaling cascades

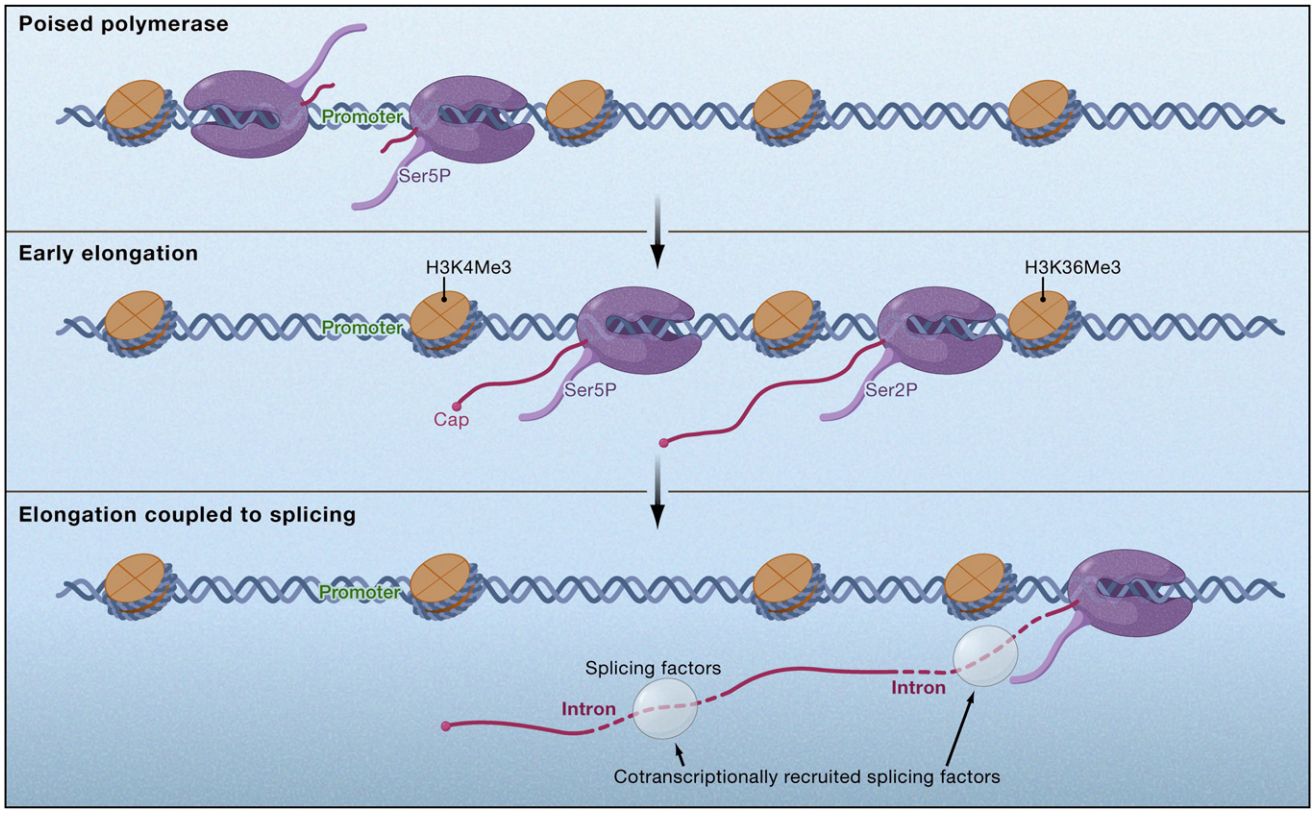

Figure 1. Transcription Elongation Is Coupled to Pre-mRNA Processing

Three stages of protein coding gene transcription are depicted. First, transcription of protein coding genes by RNA polymerase II (Pol II) involves an initial stage where the polymerase is engaged with the gene promoter, but in a poised state. Productive elongation into the body of the gene has yet to commence. These promoter-associated polymerases are shown to be associated with bidirectional transcription resulting in the production of short sense and antisense promoter transcripts (Buratowski, 2008). Second, the initially poised polymerases switch to productive transcription elongation and read into the body of the gene. This process is regulated by either developmental or cell signaling stimuli that remain largely uncharacterized. It is also associated with histone tail modifications and a switch from Pol II C-terminal domain (CTD) serine 5 phosphorylation (Ser5P) to CTD Ser2P. The initial transcript is cotranscriptionally capped by replacement of the triphosphate 5′ terminus with a 7meGppp structure. Capping enzymes are recruited to Ser5P Pol II CTD. Finally, productive transcriptional elongation is tightly coupled to cotranscriptional splicing which is facilitated by recruitment of splicing factors to the Pol II elongation complex. Nucleosomes are depicted as brown discs. Pol II is depicted in purple with a DNA channel, CTD appendage, and exiting RNA transcript. H3K4Me3, histone H3 lysine 4 trimethylation.

connected to the extracellular environment through membrane receptors (Komarnitsky et al., 2000; Peterlin and Price, 2006).

One well-characterized positive factor in higher eukaryotes is the heterodimeric protein PTEFb comprising the Cdk9 kinase and associated cyclin T (also known as CTDK1 in yeast), which generates CTD Ser2P patterns on Pol II elongating into the body of the gene (Peterlin and Price, 2006). PTEFb directly interacts with the HIV-1 early gene product Tat through its cyclin T component (Marcello et al., 2004). Tat is a key transcription elongation factor responsible for switching HIV-1 provirus from latency to full transcriptional activity. It does this by recognizing short, 5′ terminal abortive transcripts made during early proviral transcription. These short transcripts generate an RNA hairpin called TAR that directly interacts with Tat. Consequently, Tat recruits PTEFb and so efficient elongation proceeds across the proviral genome, resulting in full HIV-1 expression. Close parallels likely exist between the HIV-1 proviral promoter and the above-mentioned large set of genes that make short 5′ terminal abortive transcripts. To date, no host encoding protein factor analogous to Tat has been identified. However, it is now appreciated that PTEFb itself is tightly regulated by two negative factors, the small RNA 7SK and the protein Hexim. These form an inhibitory trimeric complex with Cdk9 effectively sequestering PTEFb away from transcription sites in the nucleus (Nguyen et al., 2001). Again, the trigger that releases Cdk9 from this repressive RNA protein complex is unknown but is likely to be associated with phosphorylation of Hexim and Cdk9 through kinases sensitive to cell signaling cascades.

It Pays to be Flexible: Transcription and Chromatin Conformation

Recent advances in microscopy have revealed that the nucleus is highly dynamic, with both genes and their associated factors showing remarkable mobility. Many inducible genes studied in yeast relocate after activation, often juxtaposed with the nuclear pore complex (NPC) (Akhtar and Gasser, 2007; Brown and Silver, 2007). This effectively allows direct injection of the gene transcript into the cytoplasm during the transcription process. Interestingly, NPC-gene association often correlates with the

capacity of some inducible genes to display transcriptional memory (Ahmed and Brickner, 2007). This term refers to the ability of a gene to reactivate more rapidly after short-term repression than when it has been repressed for longer periods. During short-term repression, the gene remains associated with the NPC so that when it is reactivated it switches into active mode more rapidly. Several features of chromatin appear to be required for transcriptional memory, including modified histone deposition (Brickner et al., 2007) and chromatin remodeling activities (Kundu et al., 2007). Also, cytoplasmic factors such as Gal1 may play a role through contacts with the cytoplasmic face of the NPC (Zacharioudakis et al., 2007). In contrast to the above analysis of the yeast nucleus, studies on nuclear organization in higher eukaryotes have led to the view that repressed heterochromatin is often associated with the nuclear lamina. This is a filamentous layer of protein that coats the nuclear face of the inner membrane. However, locus boundary elements that act to insulate active genes from repressed chromatin are also membrane associated, but in this case associate with the NPC and so may align active genes with the direct exit rout from nucleus to cytoplasm (Akhtar and Gasser, 2007).

Another characteristic feature of activated genes is that they switch their conformation when activated. In this case, a loop structure is predicted to occur in which the promoter and terminator of a gene are in close proximity (Ansari and Hampsey, 2005; O'Sullivan et al., 2004), as detected by the chromatin ligation technique called 3C (Dekker et al., 2002). Gene loop formation brings into play the 3′ terminal RNA processing mechanism. 3′ cleavage and polyadenylation of pre-mRNA are dictated by polyA signals that define the end of the mRNA. These signals are recognized by a substantial cleavage/polyadenylation protein complex (termed here polyA complex) that is recruited to CTD Ser2P through direct CTD-interacting domains (CIDs) as well as RNA binding domains (RBDs) that specifically recognize the pre-mRNA polyA signals. Specific CIDs and RBDs have been identified on individual polyA complex subunits (Bentley, 2005; Proudfoot, 2004). Some components of the polyA complex are also detected at gene promoters through interaction with general transcription factors TFIID (Dantonel et al., 1997) and TFIIB (Wu et al., 1999), presumably as a result of gene looping. Thus, mutation of particular polyA complex components (Ssu72 or Pta1) or the polyA signals themselves (particularly the conserved mammalian polyA signal, AAUAAA) causes a breakdown in gene loop structure (Perkins et al., 2008; Singh and Hampsey, 2007). Interestingly, CTD phosphorylation patterns are also correlated with gene looping. Thus, gene loop structures are lost when CTD Ser5P is prevented from forming by inactivation of Kin28 (O'Sullivan et al., 2004). Similarly, the Ssu72 protein has CTD Ser5P phosphatase activity, again pointing to a requirement for a specific CTD phosphorylation state for the formation of gene loops (Krishnamurthy et al., 2004). Although the functional significance of gene loops remains to be established, two potential roles have been proposed. First, they may act to retain Pol II once a full transcription cycle has completed, allowing it to be rechanneled back to the promoter for another round of transcription. In effect, Pol II need never release from its gene location as it is either poised on the promoter or held in a gene loop structure. Second, gene looping may play a role in gene surveillance. A first

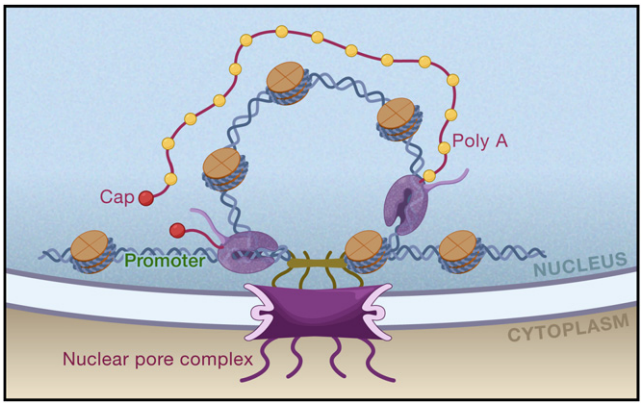

Figure 2. Transcription at the Nuclear Pore

Gene transcription by Pol II induces chromosomal movement, whereby the gene may associate with the nuclear pore complex and in so doing may form a looped conformation that brings the promoter and terminator regions into close apposition. This conformational change is illustrated by showing both the gene promoter and terminator regions and the packaged transcript associated with the nuclear pore complex in a looped conformation. Such a structure facilitates efficient nuclear export of messenger ribonucleoproteins (mRNPs). The positions of nucleosomes and Pol II (as in Figure 1) are indicated along with the packaged transcript (yellow balls depict packaging proteins).

or "pioneer" round of transcription may allow initial gene loop formation to occur, provided that authentic polyA signals are encountered by the elongating Pol II. This will then seal Pol II transcription into a continuous productive mode (Figure 2).

The chromatin template is also modified during transcription in part to enhance the efficiency of RNA synthesis and pre-mRNA processing. CTD Ser5P acts to recruit the histone methylase Set1, which trimethylates H3 lysine 4 (H3K4me3) over the promoter proximal regions (Li et al., 2007). This may aid the switch from nonproductive to productive transcriptional elongation. H3K4me3 marks also correlate with enhancement of capping as well as the recruitment of splicing factors that act on the emerging nascent RNA from the elongating Pol II complex (Sims et al., 2007). As with NPC association, H3K4me3 marks may extend through multiple rounds of transcription, affording more rapid reactivation of transcription (Ng et al., 2003). Set2 similarly trimethylates H3K36 and in this case is recruited by interaction with CTD Ser2P (Li et al., 2007; Shilatifard, 2006). Together with other elongation factors such as PAF (Rosonina and Manley, 2005), histone chaperones such as Spt6 (Yoh et al., 2007) and FACT (Belotserkovskaya and Reinberg, 2004) and the chromatin remodeler Chd1 (Sims et al., 2007), H3K36me3 marks may facilitate more efficient elongation and coupled splicing and 3′ end processing (Figure 1B). Analysis of the complex alternatively spliced mammalian gene CD44 also revealed a role for the chromatin remodeling enzyme SWI/SNF in Pol II elongation and associated splicing factor recruitment to alternatively spliced introns (Batsche et al., 2006).

Splicing and Packaging the Transcript during Pol II Elongation

The view that most pre-mRNA splicing occurs cotranscriptionally appears to prevail for all eukaryotes. In vivo studies on

spliceosome recruitment to actively transcribing genes suggests that there is an ordered association of first U1 small nuclear RNA protein complex (snRNP) to a newly formed 5′ splice site transcript followed by U2 and U5 snRNPs when the rest of the intron is synthesized (Gornemann et al., 2005; Lacadie and Rosbash, 2005). The spliceosome then forms and splicing of adjacent exons ensues. Coupled in vitro transcription:splicing systems further suggest that splicing factors are more efficiently assembled on gene templates transcribed by Pol II than on those transcribed by T7 phage RNA polymerase (Das et al., 2007). Consequently, Pol II-derived transcripts are more efficiently spliced. This was shown to be due to interaction of U1 snRNP as well as splicing regulatory proteins with Pol II CTD (Das et al., 2007). In the infrequent and relatively short introns of yeast, the advantage of cotranscriptional splicing appears less evident. However, with higher eukaryotes, the large number and often substantial size (>10 kb) of introns suggests a significant benefit for dealing with these large intronic transcripts as expeditiously as possible. One way to achieve this may be to cotranscriptionally cleave the intron sequence. It can then be degraded by exonucleases, and so long as the adjacent exons are retained on the polymerase elongation complex, then splicing can still occur. Evidence that cotranscriptional cleavage of intronic RNA can occur without affecting splicing has been demonstrated in both yeast and mammalian experimental gene systems (Dye et al., 2006; Lacadie et al., 2006). Furthermore, natural examples of cotranscriptional intronic cleavage abound, as abundant intronic pre-microRNAs are all likely to be excised from introns cotranscriptionally, yet adjacent exons in the host gene are still effectively spliced together (Kim and Kim, 2007; Morlando et al., 2008).

The direct interplay between transcription and splicing has been demonstrated in a number of studies. Over 20 years ago it was realized that the presence of a promoter-proximal intron increased transcription (Brinster et al., 1988). Subsequently, it was shown that this effect acts at least in part to enhance Pol II initiation (Furger et al., 2002). Pol II elongation is also enhanced by factors that directly interact with the splicing apparatus. Thus, the elongation factor TAT-SF1 interacts with U2 snRNP (Kameoka et al., 2004), whereas SC35, which is directly associated with splicing, promotes more efficient elongation through potential transcriptional pause sites (Lin et al., 2008). These pause sites are often found spread throughout mammalian genes. The connection between transcription and alternative splicing in mammals has also been extensively studied. In general, it is clear that where alternative splice sites compete for splicing the elongation rate of Pol II can profoundly affect the alternative splicing ratios. Pol II elongation rate is in part dictated by specific promoters, which may act to recruit a more or less processive Pol II complex (Figure 1C) (de la Mata et al., 2003).

As well as splicing the emerging Pol II transcript, the pre-mRNA must also be immediately packaged into an expanding complex with RNA binding proteins. In yeast, this packaging process is brought about by the THO/TREX complex, a multicomponent complex whose members variously function in transcription elongation, transcript-dependent recombination, and mRNA export (Iglesias and Stutz, 2008; Kohler and Hurt, 2007). Significantly, transcript packaging connects with both ends of the nuclear

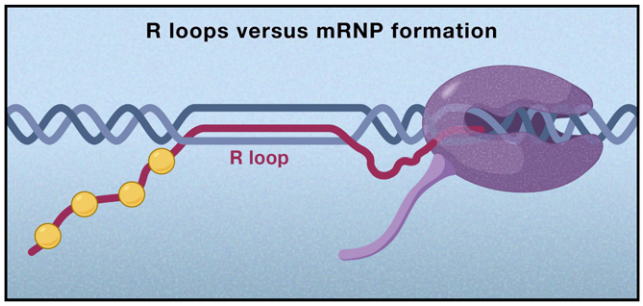

Figure 3. Fates of Transcripts Generated by Pol II Elongation
Transcripts generated by Pol II elongation have several different immediate fates. They may be cotranscriptionally packaged into messenger ribonucleoproteins (mRNPs) and after identification of splicing signals may also be cotranscriptionally spliced. Alternatively, naked transcripts may invade the DNA duplex behind elongating Pol II to form R loop structures. Here, the template DNA strand is base paired with the transcript forcing the nontemplate strand into a single-stranded conformation. Efficient mRNA packaging into mRNP particles (yellow) restricts R loop formation.

gene expression pathway. SAGA, a component of the Mediator complex required for Pol II initiation, interacts with a second complex TREX2 that in turn makes contacts with both the NPC and THO/TREX. At the other end, THO/TREX interacts with nuclear export factors such as Mex67, and these factors act to anchor messenger ribonucleoproteins (mRNPs) to the NPC (Kohler and Hurt, 2007). Nuclear surveillance pathways operate at this stage to preclude unspliced or aberrantly spliced mRNPs from exiting the nucleus. Mlp1 is a key factor associated with the NPC that is closely connected with this process (Casolari and Silver, 2004). Mutants lacking THO/TREX components show accumulated gene 3′ ends tethered to the NPC (Rougemaille et al., 2008). Also, 3′ cleavage/polyadenylation is impaired, and these aberrant NPC:mRNP associations lead to transcript destruction (Saguez et al., 2008). The principal enzyme responsible for this and most other cellular RNA turnover is the exosome. This multisubunit complex in its nuclear form contains two 3′-5′ exonucleases, Rrp6 and Dis3, which possess most of the RNase activity (Schmid and Jensen, 2008).

A key function of pre-mRNA protein packaging is to prevent the tendency of naked RNA released from the RNA exit channel from invading the DNA duplex behind the elongating Pol II. Single-strand RNA may directly base pair with the DNA template strand, thereby forcing the sense DNA into an extended single-stranded region (Figure 3). Such structures are referred to as R loops. Evidence that R loop formation is indeed a deleterious consequence of failed pre-mRNA packaging comes from studies in both yeast and mammalian cells. Mutants in the THO/TREX complex were often found to possess a marked DNA damage phenotype (Aguilera and Klein, 1990). Further characterization of specific genes in these affected mutant strains revealed elongation defects that were rescued by overexpressing RNase H or by incorporating ribozymes into the transcribed genes (Huertas and Aguilera, 2003). Both of these experiments were interpreted as indicating the presence of RNA:DNA hybrids that were predicted to slow down elongating Pol II. Similarly, the DNA damage phenotype was explicable by formation of single-strand DNA,

which is likely prone to mutagenesis. These data were confirmed in higher eukaryotic cells where depletion of serine/arginine-rich (SR) protein ASF/SF2 induced a DNA damage phenotype. The presence of single-strand DNA was also detected in these studies via bisulphite treatment of chromatin isolated from these ASF/SF2-depleted cells. This chemical treatment modifies single-strand dC residues to dU, which can then be detected by DNA sequence analysis (Li and Manley, 2005). It should be noted that pre-mRNA packaging has not been well characterized in higher eukaryotes. However, it is possible that packaging focuses on exonic regions that are thought to be covered by heterogeneous ribonucleoproteins (hnRNPs), of which SR proteins are one subclass (Bourgeois et al., 2004; Glisovic et al., 2008). Intronic sequence may be only loosely packaged with these RNA binding proteins so that R loop formation by introns is principally avoided by their removal via splicing. Consequently, the loss of splicing factors may be predicted to increase R loop formation.

Together to the End: Pre-mRNA Processing and Pol II Termination

The final stages in transcription of a gene occur when the polymerase reads through functional polyA signals, generating pre-mRNA sequences recognized by the large polyA complex. As mentioned above, this complex is recruited to elongating Pol II in part through direct interaction of its components, especially Pcf11 CID with the CTD Ser2P elongation mark (Bentley, 2005; Licatalosi et al., 2002; Proudfoot, 2004). What happens next varies depending on the type of Pol II transcribed gene as well as between different eukaryotes. In yeast, the polyA complex recognizes a series of somewhat redundant AU-rich RNA elements through direct RNA recognition domains. Successful association of polyA complex with these RNA sequences promotes transcript cleavage at the polyA site by a specific polyA complex component (CPSF-73 in higher eukaryotes or Ydh1 in yeast; Mandel et al., 2006) and subsequent polyadenylation of the newly formed 3′ terminus by Pap1, also an integral component of the polyA complex (Proudfoot, 2004). This polyadenylation process facilitates mRNA release from the transcription site and its ultimate export through the NPC to cytoplasmic translation. However, the elongating Pol II itself requires further RNA processing steps to facilitate its release from the gene template. A complex of three polypeptides, one called Rtt103, containing a CID with specificity for CTD Ser2P, is recruited to the 3′ product of 3′ end cleavage. The other component of this complex, Rat1, then acts together with its cofactor, Rai1, as a potent 5′-3′ exonuclease (often referred to as a torpedo) to degrade the nascent transcript still being generated by Pol II elongating past the polyA signal (Kim et al., 2004). Rapid degradation of this final Pol II transcript catching up with the elongating Pol II appears to be the key signal that finally stops this molecular juggernaut in its tracks.

In higher eukaryotes, a similar termination mechanism is likely to occur. However, the substantial length of genes in higher eukaryotes requires that additional termination signals in 3′ flanking regions of genes cooperate with the polyA signal to promote Pol II termination. For several characterized genes, transcription pause sites positioned just after the polyA signal act to slow down elongating Pol II (Gromak et al., 2006). This effectively provides time for the 5′-3′exonuclease Xrn2 (Rat1 homolog) to attach to the 3′ cleavage product and degrade the downstream transcript, catching up with the elongating Pol II (Kaneko et al., 2007; West et al., 2004). An interesting variation on this termination process occurs in other Pol II-transcribed genes. Here, although no pause sites are present downstream of the polyA signal, another termination element exists that is often positioned over 1 kb downstream. This termination element is associated with rapid cotranscriptional cleavage of the nascent transcript at the termination site (Dye and Proudfoot, 2001). This then provides immediate access of Xrn2 at this 3′ flanking region position, and thereby Pol II is directly terminated. Surprisingly, this termination process mediated by cotranscriptional cleavage occurs so quickly that it precedes cleavage at the polyA signal. In effect Pol II is released from the DNA template with the pre-mRNA still anchored to the CTD through contacts with the polyA complex. This final pre-mRNA processing step may therefore occur posttranscriptionally but still on a Pol II platform (West et al., 2008).

A further variation on Pol II termination has been uncovered by studies in yeast. In this case, a second Pol II termination process has been shown to operate involving yet another protein complex containing Nrd1, Nab3, and Sen1 protein components. This termination mechanism is employed by Pol II transcripts derived from snRNA or small nucleolar RNA (snoRNA) genes as well as the rapidly expanding repertoire of so-called cryptic unstable transcripts (CUTs) (Arigo et al., 2006; Steinmetz et al., 2001). A unifying feature of genes encoding snRNAs, snoRNAs, and CUTs is that they are all relatively small transcription units. The mechanism of this alternative Pol II termination process is that Nrd1 interacts with Pol II, again through a CID domain. Interestingly, this CID prefers CTD Ser5P, which neatly fits with the action of this termination mechanism on shorter Pol II gene transcripts (Gudipati et al., 2008; Vasiljeva et al., 2008). Termination signals for this alternative mechanism are redundant GUAA/G and UCUU sequences recognized by RNA binding domains of Nrd1 and Nab3, respectively. An unresolved issue for this termination process is how these RNA signals remain accessible for Nrd1 and Nab3 rather than being packaged into mRNPs by the action of THO/TREX. Indeed, this leads to the question of how promoter-proximal Nrd1-dependent termination is prevented in regular protein coding genes that need to read through the whole (usually longer gene) to the regular cleavage/polyA signals where the polyA complex followed by Rat1 and associated factors promote Pol II termination.

This final stage in pre-mRNA cotranscriptional processing is both a key step in efficient gene expression and a critical control point in preventing aberrant gene expression. When 3′ processing/polyadenylation is either inefficient or compromised by gene mutation, then the nuclear exosome is recruited to rapidly degrade the unwanted transcript. Interestingly, the Nrd1 termination pathway achieves this process by directly recruiting the exosome through interaction with Nrd1 (Vasiljeva and Buratowski, 2006). In effect, Nrd1-mediated Pol II termination is associated with rapid transcript degradation. snRNAs and snoRNAs escape this exosome-mediated degradation by protecting the mature RNAs cotranscriptionally with specific RNA binding

proteins (Morlando et al., 2004). In contrast, CUTs have no such protection and are rapidly degraded. It should also be mentioned that the above described mechanisms of Pol II termination must somehow be reconciled with the phenomena of gene loop formation and gene 3′ end association with the NPC. Both of these mechanisms require functional 3′ end processing and termination signals. It therefore seems plausible that Pol II termination does not simply release Pol II into the nucleoplasm but rather that terminated Pol II is retained at specific nuclear locations that allow its rapid reassociation with gene promoters.

Cytoplasmic Events

In addition to these interconnected events in the nucleus, there are also well-documented connections between nuclear processes and downstream cytoplasmic events. This influence is mediated by the repertoire of proteins that, together with the mRNA, make up the mRNP (Glisovic et al., 2008; Moore, 2005). Many mRNP proteins first encounter the transcript as it emerges from Pol II in the nucleus and then accompany the processed mRNA out to the cytoplasm (Figure 4). mRNP proteins can be thought of as adaptors that add functionality to a transcript by interfacing with a wide variety of cellular machineries. Some of these adaptors recognize particular structures (such as the 5′ cap or the 3′ polyA tail) or short consensus sequences, whereas others become associated primarily as a consequence of the mRNA manufacturing process. Of particular note is the splicing history of an mRNA, which can influence almost every stage of its subsequent metabolism, including how quickly and efficiently it is exported from the nucleus, whether it is correctly localized in the cytoplasm, how efficiently it is taken up by the translation machinery, and whether it is a target for nonsense-mediated mRNA decay (NMD) (Giorgi and Moore, 2007; Le Hir et al., 2003).

The Exon Junction Complex and Other Molecular Graffiti

To date a surprising number of mRNP proteins have been shown to exhibit some degree of splicing dependence in their association with mRNA (Merz et al., 2007). One of the most extensively studied is the exon junction complex (EJC), a set of proteins stably deposited on spliced mRNAs about 20 nucleotides upstream of exon-exon junctions (Le Hir et al., 2000). That such a complex might exist was initially intimated by the then unexpected observation that recognition of termination codons as premature in mammalian mRNAs depends on their spatial relationship to the 3′-most exon-exon junction. This suggested that the splicing machinery was capable of tagging splice junctions with some sort of mark that could signify to downstream processes the positions at which introns had resided in the original transcript (Thermann et al., 1998; Zhang et al., 1998). We now know that this molecular tag consists of a tri- or tetrameric protein core stably deposited on the splice donor exon at a late stage in the splicing process (Le Hir and Andersen, 2008). This core accompanies the mRNA to the cytoplasm, where EJCs within open reading frames (ORFs) are removed by the first or "pioneer" round of translation (Dostie and Dreyfuss, 2002; Lejeune et al., 2002). In both the nucleus and cytoplasm, other factors that associate more dynamically interface with the EJC core to mediate its widespread effects on mRNA metabolism (Tange et al., 2004).

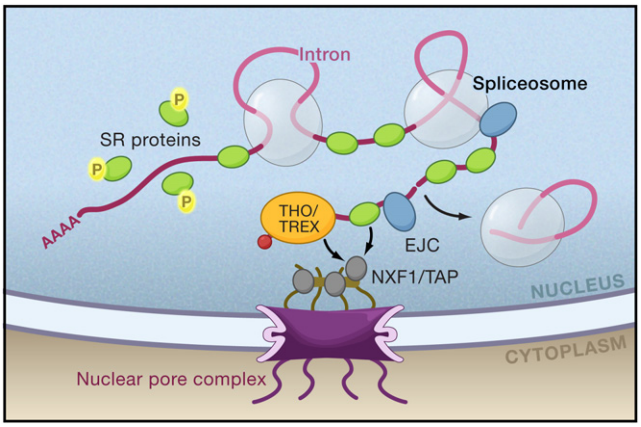

Figure 4. Splicing Factors Contribute to mRNP Export

Messenger ribonucleoprotein (mRNP) components associated with splicing contribute to mRNA export in mammalian cells. Both the THO/TREX complex and the exon junction complex (EJC) are loaded onto mRNAs as a consequence of splicing. Splicing-dependent hypophosphorylation of shuttling SR proteins may also stabilize their association with mRNA. Both hypophosphorylated SR proteins and the THO/TREX complex can act as export adaptors via interaction with the nuclear export receptor NXF1/TAP. Positioning of the THO/TREX complex near the 5′ cap may assist mRNAs in exiting the nucleus 5′ end first to more efficiently engage the cytoplasmic translation machinery.

The mystery of how the EJC core remains stably bound to spliced mRNAs without benefit of either covalent attachment or significant structure or sequence specificity was solved by identification of the DEAD-box protein eIF4AIII (DDX48) as the main RNA anchoring constituent (Shibuya et al., 2004). DEAD-box proteins, a subgroup of the DExH/D-box family of RNA-dependent ATPases, are often presumed to function as RNA or RNP remodelers on the basis of their sequence similarity to the SF2 family of DNA helicases (Cordin et al., 2006; Jankowsky and Fairman, 2007). But within the EJC core, inhibition of eIF4AIII ATPase activity by its binding partners Y14 and Magoh locks eIF4AIII into a stable RNA binding configuration. An additional core protein, MLN51, increases the affinity of eIF4AIII for RNA, which is otherwise low (Ballut et al., 2005). Thus, as a component of the EJC, eIF4AIII functions not as a helicase or translocase, but rather as a regulatable, sequence-independent RNA binding protein. This "clothes peg"-like activity of eIF4AIII both preserves the synthetic history of the mRNA and adds functionality to the mRNP as a binding platform for other key regulatory factors. Intriguingly, two other DEAD-box proteins, DDX3 and DDX5/p68, were recently shown to associate with spliced mRNAs in a manner similar to the EJC (that is, their association required both splicing and the EJC binding region about 20 nucleotides upstream of the splice junction) (Merz et al., 2007). Like the EJC proteins, DDX3 and DDX5/p68 have been functionally implicated in diverse processes including transcription, splicing, mRNA export, translation, and mRNA decay (Fuller-Pace and Ali, 2008; Rosner and Rinkevich, 2007). Currently, it is unknown whether these new DEAD-box proteins act in concert with eIF4AIII-containing EJCs or whether they are themselves anchors for alternate EJC-like complexes. Supporting the latter notion is evidence that the recruitment and/or function of some

splicing-dependent mRNP proteins can occur independently of eIF4AIII (Gehring et al., 2005; Zhang and Krainer, 2007).

Other mRNP components with direct links to splicing include the shuttling serine/arginine-rich (SR) proteins. These proteins join pre-mRNAs cotranscriptionally and generally recognize short consensus sequences by way of one or two N-terminal RNA recognition motifs (RRMs). A characteristic C-terminal domain rich in arginine/serine dipeptides (the RS domain) can serve either as a protein-protein or a protein-RNA interface and is subject to dynamic serine phosphorylation (Bourgeois et al., 2004; Huang and Steitz, 2005). In contrast to the EJC, which has no apparent role in splicing (Shibuya et al., 2004; Zhang and Krainer, 2007), SR proteins are key players in recruiting the splicing machinery to constitutive splice sites, in spliceosome assembly and in directing alternative splicing (see Review by M.C. Wahl, C.L. Will, and R. Luhrmann on page 701 of this issue). Dependent on their binding site context within a particular transcript (that is, intronic or exonic), their posttranslational modification state, and the levels of other splicing regulators in that cell type, various SR proteins can act to either enhance or inhibit splicing of nearby exons and splice sites (Bourgeois et al., 2004). Upon completion of pre-mRNA processing, those SR proteins bound to exonic sequences can then accompany the mRNA to the cytoplasm where, like the EJC, they can direct mRNA localization, translation, and decay (Huang and Steitz, 2005). Further paralleling the EJC, several SR proteins have recently been shown to exhibit some degree of splicing-dependent mRNP recruitment when splicing is uncoupled from transcription in vitro (Merz et al., 2007).

Splicing and Subcellular mRNA Localization

As discussed above, a key mediator of nuclear mRNA export is the THO/TREX complex. This assemblage principally consists of the tetra- (budding yeast) or pentameric (metazoans) THO complex, which functions in transcription elongation and transcript-dependent recombination, plus the mRNA export factors Yra1 (REF/Aly in mammals) and Sub2 (UAP56 in mammals). Yra1 is an RRM-containing RNA binding protein that bridges the mRNA to the export receptor Mex67 (NXF1/TAP in mammals), whereas Sub2 is a DEAD-box protein that also functions in spliceosome assembly (Iglesias and Stutz, 2008; Kohler and Hurt, 2007; Shen et al., 2008). In budding yeast, where most genes lack introns, the THO/TREX complex is closely associated with the transcription machinery, where it is thought to coat the length of intronless transcripts with Yra1 and Sub2 as the RNA emerges from the elongating polymerase (Abruzzi et al., 2004). In mammals, however, where most genes contain multiple introns, REF/Aly and UAP56 appear to be recruited more as a consequence of splicing than of transcription. In vivo, both proteins colocalize with transcription sites for intron-containing genes but not with mutant versions incapable of splicing (Custodio et al., 2004). Further, when uncoupled from transcription in vitro, THO/TREX complex recruitment is strongly 5′ cap and splicing dependent (Cheng et al., 2006; Masuda et al., 2005). Although REF/Aly and UAP56 were originally thought to be recruited via the EJC, more recent evidence indicates that these proteins primarily reside on the region immediately downstream of the cap, tethered there by an interaction between REF/Aly and the nuclear cap binding protein CBP80 (Cheng et al., 2006). Thus, unlike the EJC, which is presumably deposited at every exon-exon junction, the mammalian THO/TREX complex appears to associate most strongly with the 5′-most exon of spliced mRNAs. These findings nicely reconcile a long-standing conundrum that the cap facilitates the export of spliced mRNAs but is of lesser consequence for intronless mRNAs (Masuyama et al., 2004). Whereas REF/Aly is not essential for bulk mRNA export in metazoans (Gatfield and Izaurralde, 2002; Longman et al., 2003), its recruitment via splicing can clearly increase the speed and efficiency of the export process (Luo and Reed, 1999; Valencia et al., 2008). Furthermore, the positioning of the THO/TREX complex at the 5′-end of spliced mRNAs has been proposed to impart directionality so that mRNAs emerge from the nuclear pore 5′-end first to more efficiently engage the translation machinery (Valencia et al., 2008).

In addition to the THO/TREX complex, SR and SR-like proteins can also function as mRNA export adaptors (Huang and Steitz, 2005). As with REF/Aly, the shuttling SR proteins SRp20, 9G8 and ASF/SF2 can all serve as mRNP binding sites for the general export receptor NXF1/TAP. Intriguingly, NXF1/TAP preferentially interacts with the shuttling SR proteins in their hypophosphorylated state, the state thought to be active for mRNA export. Conversely, SR proteins are initially recruited to pre-mRNAs for splicing in a hyperphosphorylated state, and become partially dephosphorylated as the splicing reaction proceeds. Thus, it has been suggested that the export competence of the spliced mRNP is signaled by the phosphorylation status of its bound SR proteins (Huang and Steitz, 2005; Kohler and Hurt, 2007). Given recent evidence that some SR proteins (including 9G8 and ASF/SF2) exhibit splicing-dependent mRNP recruitment in vitro (Merz et al., 2007), perhaps their partial dephosphorylation during splicing also serves to stabilize their interaction with exonic regions, enabling them to remain associated with the spliced mRNP. After export, rephosphorylation of the SR domains is thought to trigger their release from the mRNA and facilitate their reimport into the nucleus (Huang and Steitz, 2005; Kohler and Hurt, 2007). Such a cycle of nuclear dephosphorylation-dependent mRNA and export receptor binding and cytoplasmic rephosphorylation-dependent mRNA and export receptor release has been well documented for the SR-like mRNA export adaptor Npl3 in budding yeast (Gilbert and Guthrie, 2004).

Once released from the nuclear pore, many mRNAs are further localized to particular sites in the cytoplasm (Besse and Ephrussi, 2008; see Review by K.C. Martin and A. Ephrussi on page 719 of this issue). A recent genome-wide study of over 2300 mRNAs in *Drosophila* embryos revealed that a remarkable 71% of these exhibited a distinct localization pattern. Further, tight colocalization of encoded proteins with their mRNAs suggests that subcellular protein localization patterns are largely driven by mRNA localization (Lecuyer et al., 2007). One localized *Drosophila* mRNA that has been particularly well-studied is *oskar*. *oskar* mRNA is produced by nurse cell nuclei and imported into the developing oocyte, where it is subsequently localized to the posterior pole. Restricted translation of *oskar* protein at this pole is crucial for early pattern formation in the developing embryo. Among factors required for *oskar* mRNA transport are

the EJC core factors eIF4AIII, Tsunagi, Mago Nashi, and Barentz (the *Drosophila* orthologs of Y14, Magoh, and MLN51, respectively) (Palacios, 2002; Palacios et al., 2004), and splicing of the first intron in *oskar* pre-mRNA is essential for subsequent mRNA localization (Hachet and Ephrussi, 2004). Consistent with this, all four EJC core factors initially accumulate with *oskar* at the posterior pole (Palacios, 2002; Palacios et al., 2004). Because *oskar*'s first intron is within the ORF, its associated EJC is subject to removal by the pioneer round of translation (Dostie and Dreyfuss, 2002; Lejeune et al., 2002). This indicates that *oskar* experiences no pioneer round until it reaches its final destination, consistent with the idea that mRNAs are maintained in a translationally quiescent state during the localization process (Besse and Ephrussi, 2008; Giorgi and Moore, 2007). What remains to be resolved is the exact role of the EJC in *oskar* localization—is it necessary for translational silencing, or does it participate more directly as part of the transport machinery? Also, *oskar* is the only mRNA to date whose localization is known to have a clear splicing dependence. Therefore, whether the EJC is a general player in mRNA localization remains to be seen.

Splicing Makes for Better Translation

Another well-documented effect of splicing on cytoplasmic mRNA metabolism is enhanced translational efficiency (Le Hir et al., 2003; Le Hir and Seraphin, 2008). That is, spliced mRNAs on average yield more protein molecules than do otherwise identical cDNA transcripts. This is because spliced mRNAs more efficiently engage the translation machinery during the pioneer round of translation, such that a greater percentage of spliced mRNA molecules end up associated with polysomes than do unspliced mRNAs. One advantage of this phenomenon is that it favors the translational uptake of newly made mRNAs that are still associated with their nuclear-acquired proteins over older transcripts that have already been translated and have therefore lost these components. This effect may shorten the lag time between transcriptional induction and protein expression, which could be particularly important for signal transduction pathways triggering new mRNA synthesis.

A recent flurry of papers has begun to sort out the previously mysterious means by which the EJC serves to enhance translation initiation (Figure 5A). One proposed mechanism involves a bridge between the EJC and the 48S preinitiation complex mediated by the Y14:Magoh binding protein PYM (Diem et al., 2007). Another is through EJC-dependent recruitment of the 40S ribosomal protein S6 kinase 1 (S6K1) (Ma et al., 2008). S6K1 is a central player in the TOR signaling cascade, a major regulator of protein expression related to cell growth (Bhaskar and Hay, 2007). When activated by the TOR pathway, S6K1 enhances translation initiation by activating stimulatory and inactivating inhibitory factors bound at and around the 5′ cap of TOR target mRNAs. New work from Blenis and colleagues has revealed that activated S6K1 is specifically recruited to newly synthesized mRNAs via EJC-bound SKAR (a S6K1 target), where it promotes the pioneer round of translation (Ma et al., 2008). Supporting this idea that the TOR pathway is a general player in the preferential translation of mRNPs still associated with their nuclear-acquired factors, Cáceres and coworkers recently demonstrated that the shuttling SR protein ASF/SF2

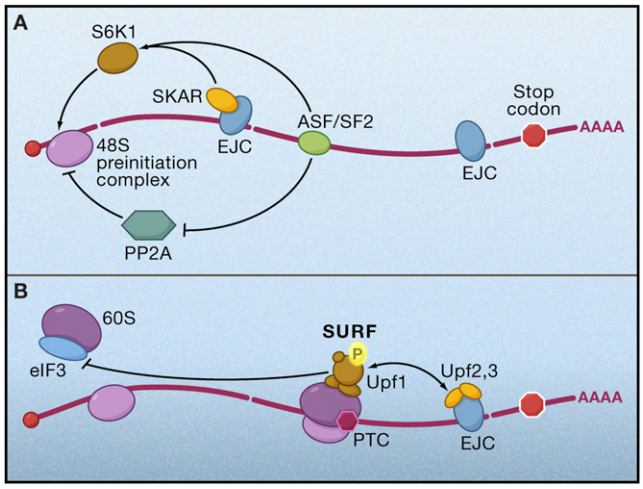

Figure 5. Splicing Factors Regulate Translation Initiation

Effects of splicing-dependent mRNP components on translation initiation

(A) Both the exon junction complex (EJC) and ASF/SF2 bound to spliced mRNA can promote the first or "pioneer" round of translation by recruiting 40S ribosomal protein S6 kinase 1 (S6K1), a component of the TOR signaling cascade. For the EJC pathway, this is accomplished via the EJC-interacting protein SKAR. ASF/SF2 can also promote translation initiation by inhibiting the S6K1 antagonist PP2A phosphatase.

(B) When an EJC is located more than 50 nucleotides downstream of a premature termination codon (PTC), interaction between the EJC and SURF complex causes phosphorylation of Upf1, which then inhibits additional rounds of translation by an interaction with the translation initiation factor eIF3.

also enhances translation initiation via recruitment of S6K1 (Michlewski et al., 2008). Like the EJC, ASF/SF2 had previously been shown to enhance translation of bound mRNAs, thereby providing another link between splicing and translation (Sanford et al., 2004). In addition to S6K1, ASF/SF2 also interacts with PP2A phosphatase, an antagonist of S6K1-dependent phosphorylation. An attractive model, which remains to be tested, is that ASF/SF2 plays a dual role in promoting translation initiation by both recruiting activated S6K1 and inhibiting factor dephosphorylation by PP2A (Michlewski et al., 2008).

Splicing and NMD: Not All Nonsense

One of the strongest connections between nuclear and cytoplasmic mRNA metabolism is the link between pre-mRNA splicing and nonsense-mediated mRNA decay (NMD). NMD is a translation-dependent degradation pathway specifically targeting mRNAs wherein the first inframe stop codon is in a poor context for translation termination. In mammals, the presence of one or more EJCs 50 or more nucleotides downstream of such a stop codon can greatly enhance the efficiency of NMD (Chang et al., 2007; Stalder and Muhlemann, 2008). Thus, in the vast majority of mammalian genes, the constitutive stop codon is either in the last exon or within 50 nucleotides of the final exon-exon junction (Nagy and Maquat, 1998). The means by which the EJC enhances NMD is by serving as a binding platform for the NMD-specific factors Upf2 and Upf3. The central player in this process is the RNA helicase Upf1, which forms a surveillance or SURF (Smg1-Upf1-eRF1-eRF3) complex with its cognate kinase, Smg1, and

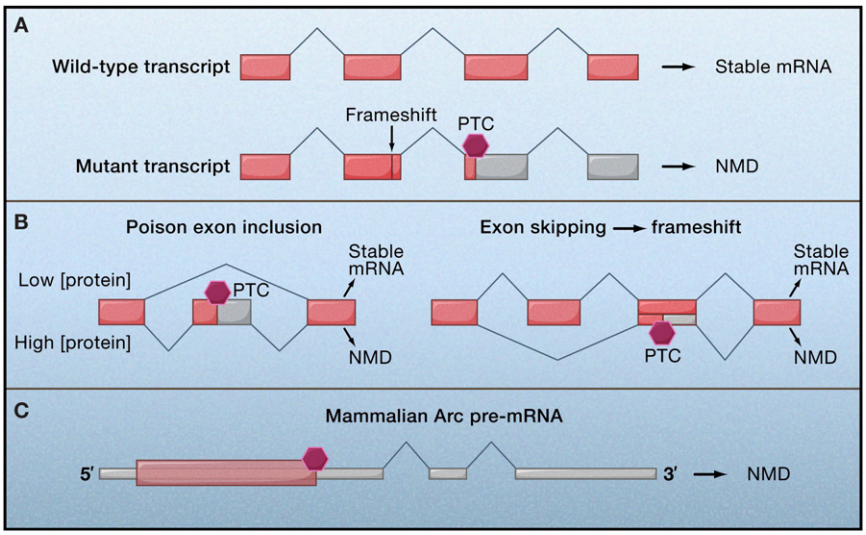

Figure 6. Splicing Patterns of Various NMD Substrates

(A) One function of nonsense mediated decay (NMD) is to eliminate mutant mRNAs containing a truncated open reading frame (red bar).

(B) Examples of alternative splicing patterns used to regulate gene expression via NMD. At low concentrations of the encoded protein, the default splicing pattern (top) results in a full-length open reading frame and stable protein expression. However, when the encoded protein concentration becomes too high, it alters splicing of its own message (bottom) to include a PTC. Inclusion of a "poison exon" that introduces a PTC is typical of SR proteins, which are usually splicing activators, whereas exon skipping is typical of heterogeneous ribonucleoproteins (hnRNPs), which tend to act as splicing repressors.

(C) The mammalian *Arc* gene contains two introns downstream of its normal stop codon, making the constitutively spliced mRNA a natural NMD target.

the release factors eRF1 and eRF3 bound to the ribosome stalled at the stop codon. Contact between the SURF complex and Upf2 bound to a downstream EJC triggers phosphorylation of Upf1 by SMG1 (Kashima et al., 2006). By a mechanism yet to be elucidated, phosphorylated Upf1 promotes recruitment of the RNA decay machinery, thus targeting the bound RNA for rapid degradation. But, even before the mRNA is eliminated, phosphorylated Upf1 apparently inhibits further rounds of translation by preventing conversion of 48S preinitiation complexes into 80S ribosomes through interaction with eIF3 (Isken et al., 2008). Thus, when positioned downstream of a stop codon, the EJC has the opposite effect on protein expression from when it is situated in the 5′ untranslated region (UTR) or ORF (Figure 5B). Conversely, the efficiency of NMD is also affected by the efficiency of such upstream splicing events (Gudikote et al., 2005). Consistent with their roles in facilitating translation of newly synthesized mRNAs (see above), both the EJC and ASF/SF2 have been implicated in this additional splicing-dependent enhancement of NMD (Gudikote et al., 2005; Zhang and Krainer, 2004). Indeed, it has been suggested that efficient splicing and EJC deposition evolved to enhance both protein production and mRNA surveillance (Gudikote et al., 2005).

NMD was originally described as a means for eliminating mutant or malformed mRNAs containing unnatural or premature termination codons (PTCs) (Figure 6A). Such mRNAs are produced from mutant alleles containing a frameshift or nonsense codon produced for example by unproductive rearrangements at V(D)J loci in B and T cells or from errors introduced into individual mRNA molecules by the transcription and pre-mRNA processing machineries (Chang et al., 2007; Isken and Maquat, 2007). Indeed, such mutant alleles account for up to 30% of all human hereditary disorders (Holbrook et al., 2004). In some cases, such as the *unc-54* myosin heavy chain gene in the nematode *C. elegans*, it has been well documented that the C-terminally truncated proteins encoded by PTC-containing alleles can exert dominant negative effects if the NMD pathway is genetically disabled (Pulak and Anderson, 1993). Thus, by suppressing expression of potentially deleterious truncated proteins, NMD provides an important clean up and quality control function. Additionally, by eliminating unproductively spliced mRNAs, NMD may allow organisms to continually sample new alternative splice forms, some of which could have beneficial activities on an evolutionary timescale.

Yet, this clean up and mRNA quality control function is far from NMD's sole role in the cell. Recent evidence indicates that NMD is also a key player in the posttranscriptional regulation of wild-type genes (Stalder and Muhlemann, 2008). For example, numerous pre-mRNA splicing factors appear to regulate their own expression by targeting their mRNAs to NMD when intracellular protein concentrations become too high. This is typically accomplished by altering the pre-mRNA splicing pattern, such that the alternatively spliced mRNA carries an EJC downstream of the stop codon and so is an NMD target (Figure 6B) (McGlincy and Smith, 2008). In this new twist on a classic self-regulatory feedback loop, the combination of alternative splicing and NMD functions to maintain cellular protein concentrations within an optimal range. Indeed, some of the more global alternative splicing changes observable in cells wherein NMD has been inhibited most likely result from pleiotropic effects induced by misregulation of key alternative splicing factors in this way.

Other mRNAs are natural NMD targets due to constitutively spliced introns in their 3′ UTRs (Figure 6C). One such example is Arc/Arg3.1 (activity-regulated cytoskeletal-associated protein/activity-regulated gene 3.1), an immediate early gene whose expression is rapidly induced upon neuronal activity. Arc protein serves as a key modulator of multiple forms of neuronal plasticity and is essential for the consolidation of long-term memory. Upon transcriptional induction, Arc mRNA is trafficked to dendrites where it is locally translated at activated synapses. In mammals, the Arc gene has two conserved introns in its 3′ UTR, and Arc mRNA exhibits translation-dependent decay via the Upf1 pathway. Interference with EJC deposition leads to increased levels of Arc protein at synapses and quantifiable changes in synaptic scaling (Giorgi et al., 2007). Arc thus illustrates another use of NMD to regulate gene expression. Because NMD substrates are stable as long as they remain translationally

quiescent, such mRNAs can accumulate at specific sites in the cytoplasm where they may be poised for rapid response to external stimuli. Upon translational activation, rapid NMD onset could quickly curtail protein synthesis, resulting in a tightly controlled burst of the desired product. Indeed, given that mammalian NMD is thought to occur coincident with the pioneer round of translation (Isken and Maquat, 2007), constitutive NMD targets potentially encode just a single polypeptide prior to their demise. Thus, unlike the cases above where NMD of alternatively spliced mRNAs is exploited to lower mRNA and protein levels in bulk, activation and degradation of constitutive NMD targets may serve as a means to produce tightly controlled bursts of protein at distinct subcytoplasmic locations (Giorgi et al., 2007).

Conclusion

This review attempts to chart the amazingly complex process of gene expression in eukaryotes. This begins with pre-mRNA synthesis from an active gene in nuclear chromatin and ends with degradation of the mature mRNA at often distant sites of protein synthesis in the cytoplasm. It is increasingly clear that enormous trouble is taken to both streamline this process for productive gene expression and to quickly eliminate erroneous mRNAs that might otherwise result in molecular disaster. At every point along the way, multifunctional proteins and RNP complexes facilitate communication between upstream and downstream steps, providing both feedforward and feedback information essential for proper coordination of what can only be described as an intricate and astonishing web of regulation.

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REFERENCES

Abruzzi, K.C., Lacadie, S., and Rosbash, M. (2004). Biochemical analysis of TREX complex recruitment to intronless and intron-containing yeast genes. EMBO J. *23*, 2620–2631.

Aguilera, A., and Klein, H.L. (1990). HPR1, a novel yeast gene that prevents intrachromosomal excision recombination, shows carboxy-terminal homology to the Saccharomyces cerevisiae TOP1 gene. Mol. Cell. Biol. *10*, 1439–1451.

Ahmed, S., and Brickner, J.H. (2007). Regulation and epigenetic control of transcription at the nuclear periphery. Trends Genet. *23*, 396–402.

Akhtar, A., and Gasser, S.M. (2007). The nuclear envelope and transcriptional control. Nat. Rev. Genet. *8*, 507–517.

Ansari, A., and Hampsey, M. (2005). A role for the CPF 3′-end processing machinery in RNAP II-dependent gene looping. Genes Dev. *19*, 2969–2978.

Arigo, J.T., Eyler, D.E., Carroll, K.L., and Corden, J.L. (2006). Termination of cryptic unstable transcripts is directed by yeast RNA-binding proteins Nrd1 and Nab3. Mol. Cell *23*, 841–851.

Ballut, L., Marchadier, B., Baguet, A., Tomasetto, C., Seraphin, B., and Le Hir, H. (2005). The exon junction core complex is locked onto RNA by inhibition of eIF4AIII ATPase activity. Nat. Struct. Mol. Biol. *12*, 861–869.

Batsche, E., Yaniv, M., and Muchardt, C. (2006). The human SWI/SNF subunit Brm is a regulator of alternative splicing. Nat. Struct. Mol. Biol. *13*, 22–29.

Belotserkovskaya, R., and Reinberg, D. (2004). Facts about FACT and transcript elongation through chromatin. Curr. Opin. Genet. Dev. *14*, 139–146.

Bentley, D.L. (2005). Rules of engagement: co-transcriptional recruitment of pre-mRNA processing factors. Curr. Opin. Cell Biol. *17*, 251–256.

Besse, F., and Ephrussi, A. (2008). Translational control of localized mRNAs: restricting protein synthesis in space and time. Natl. Rev. *9*, 971–980.

Bhaskar, P.T., and Hay, N. (2007). The two TORCs and Akt. Dev. Cell *12*, 487–502.

Bourgeois, C.F., Lejeune, F., and Stevenin, J. (2004). Broad specificity of SR (serine/arginine) proteins in the regulation of alternative splicing of pre-messenger RNA. Prog. Nucleic Acid Res. Mol. Biol. *78*, 37–88.

Brickner, D.G., Cajigas, I., Fondufe-Mittendorf, Y., Ahmed, S., Lee, P.C., Widom, J., and Brickner, J.H. (2007). H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. PLoS Biol. *5*, e81.

Brinster, R.L., Allen, J.M., Behringer, R.R., Gelinas, R.E., and Palmiter, R.D. (1988). Introns increase transcriptional efficiency in transgenic mice. Proc. Natl. Acad. Sci. USA *85*, 836–840.

Brown, C.R., and Silver, P.A. (2007). Transcriptional regulation at the nuclear pore complex. Curr. Opin. Genet. Dev. *17*, 100–106.

Buratowski, S. (2008). Transcription. Gene expression–where to start? Science *322*, 1804–1805.

Casolari, J.M., and Silver, P.A. (2004). Guardian at the gate: preventing unspliced pre-mRNA export. Trends Cell Biol. *14*, 222–225.

Chang, Y.F., Imam, J.S., and Wilkinson, M.F. (2007). The nonsense-mediated decay RNA surveillance pathway. Annu. Rev. Biochem. *76*, 51–74.

Cheng, H., Dufu, K., Lee, C.S., Hsu, J.L., Dias, A., and Reed, R. (2006). Human mRNA export machinery recruited to the 5′ end of mRNA. Cell *127*, 1389–1400.

Cordin, O., Banroques, J., Tanner, N.K., and Linder, P. (2006). The DEAD-box protein family of RNA helicases. Gene *367*, 17–37.

Core, L.J., and Lis, J.T. (2008). Transcription regulation through promoter-proximal pausing of RNA polymerase II. Science *319*, 1791–1792.

Cramer, P. (2004). RNA polymerase II structure: from core to functional complexes. Curr. Opin. Genet. Dev. *14*, 218–226.

Custodio, N., Carvalho, C., Condado, I., Antoniou, M., Blencowe, B.J., and Carmo-Fonseca, M. (2004). In vivo recruitment of exon junction complex proteins to transcription sites in mammalian cell nuclei. RNA *10*, 622–633.

Dantonel, J.C., Murthy, K.G., Manley, J.L., and Tora, L. (1997). Transcription factor TFIID recruits factor CPSF for formation of 3′ end of mRNA. Nature *389*, 399–402.

Das, R., Yu, J., Zhang, Z., Gygi, M.P., Krainer, A.R., Gygi, S.P., and Reed, R. (2007). SR proteins function in coupling RNAP II transcription to pre-mRNA splicing. Mol. Cell *26*, 867–881.

de la Mata, M., Alonso, C.R., Kadener, S., Fededa, J.P., Blaustein, M., Pelisch, F., Cramer, P., Bentley, D., and Kornblihtt, A.R. (2003). A slow RNA polymerase II affects alternative splicing in vivo. Mol. Cell *12*, 525–532.

Dekker, J., Rippe, K., Dekker, M., and Kleckner, N. (2002). Capturing chromosome conformation. Science *295*, 1306–1311.

Diem, M.D., Chan, C.C., Younis, I., and Dreyfuss, G. (2007). PYM binds the cytoplasmic exon-junction complex and ribosomes to enhance translation of spliced mRNAs. Nat. Struct. Mol. Biol. *14*, 1173–1179.

Dostie, J., and Dreyfuss, G. (2002). Translation is required to remove Y14 from mRNAs in the cytoplasm. Curr. Biol. *12*, 1060–1067.

Dye, M.J., and Proudfoot, N.J. (2001). Multiple transcript cleavage precedes polymerase release in termination by RNA polymerase II. Cell *105*, 669–681.

Dye, M.J., Gromak, N., and Proudfoot, N.J. (2006). Exon tethering in transcription by RNA polymerase II. Mol. Cell *21*, 849–859.

Egloff, S., and Murphy, S. (2008). Cracking the RNA polymerase II CTD code. Trends Genet. *24*, 280–288.

Fuller-Pace, F.V., and Ali, S. (2008). The DEAD box RNA helicases p68 (Ddx5) and p72 (Ddx17): novel transcriptional co-regulators. Biochem. Soc. Trans. *36*, 609–612.

Furger, A., O'Sullivan, J.M., Binnie, A., Lee, B.A., and Proudfoot, N.J. (2002). Promoter proximal splice sites enhance transcription. Genes Dev. *16*, 2792–2799.

Gatfield, D., and Izaurralde, E. (2002). REF1/Aly and the additional exon junction complex proteins are dispensable for nuclear mRNA export. J. Cell Biol. *159*, 579–588.

Gehring, N.H., Kunz, J.B., Neu-Yilik, G., Breit, S., Viegas, M.H., Hentze, M.W., and Kulozik, A.E. (2005). Exon-junction complex components specify distinct routes of nonsense-mediated mRNA decay with differential cofactor requirements. Mol. Cell *20*, 65–75.

Gilbert, W., and Guthrie, C. (2004). The Glc7p nuclear phosphatase promotes mRNA export by facilitating association of Mex67p with mRNA. Mol. Cell *13*, 201–212.

Giorgi, C., and Moore, M.J. (2007). The nuclear nurture and cytoplasmic nature of localized mRNPs. Semin. Cell Dev. Biol. *18*, 186–193.

Giorgi, C., Yeo, G.W., Stone, M.E., Katz, D.B., Burge, C., Turrigiano, G., and Moore, M.J. (2007). The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. Cell *130*, 179–191.

Glisovic, T., Bachorik, J.L., Yong, J., and Dreyfuss, G. (2008). RNA-binding proteins and post-transcriptional gene regulation. FEBS Lett. *582*, 1977–1986.

Gornemann, J., Kotovic, K.M., Hujer, K., and Neugebauer, K.M. (2005). Cotranscriptional spliceosome assembly occurs in a stepwise fashion and requires the cap binding complex. Mol. Cell *19*, 53–63.

Gromak, N., West, S., and Proudfoot, N.J. (2006). Pause sites promote transcriptional termination of mammalian RNA polymerase II. Mol. Cell. Biol. *26*, 3986–3996.

Gudikote, J.P., Imam, J.S., Garcia, R.F., and Wilkinson, M.F. (2005). RNA splicing promotes translation and RNA surveillance. Nat. Struct. Mol. Biol. *12*, 801–809.

Gudipati, R.K., Villa, T., Boulay, J., and Libri, D. (2008). Phosphorylation of the RNA polymerase II C-terminal domain dictates transcription termination choice. Nat. Struct. Mol. Biol. *15*, 786–794.

Guenther, M.G., Levine, S.S., Boyer, L.A., Jaenisch, R., and Young, R.A. (2007). A chromatin landmark and transcription initiation at most promoters in human cells. Cell *130*, 77–88.

Hachet, O., and Ephrussi, A. (2004). Splicing of oskar RNA in the nucleus is coupled to its cytoplasmic localization. Nature *428*, 959–963.

Holbrook, J.A., Neu-Yilik, G., Hentze, M.W., and Kulozik, A.E. (2004). Nonsense-mediated decay approaches the clinic. Nat. Genet. *36*, 801–808.

Huang, Y., and Steitz, J.A. (2005). SRprises along a messenger's journey. Mol. Cell *17*, 613–615.

Huertas, P., and Aguilera, A. (2003). Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. Mol. Cell *12*, 711–721.

Iglesias, N., and Stutz, F. (2008). Regulation of mRNP dynamics along the export pathway. FEBS Lett. *582*, 1987–1996.

Isken, O., and Maquat, L.E. (2007). Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function. Genes Dev. *21*, 1833–1856.

Isken, O., Kim, Y.K., Hosoda, N., Mayeur, G.L., Hershey, J.W., and Maquat, L.E. (2008). Upf1 phosphorylation triggers translational repression during nonsense-mediated mRNA decay. Cell *133*, 314–327.

Jankowsky, E., and Fairman, M.E. (2007). RNA helicases–one fold for many functions. Curr. Opin. Struct. Biol. *17*, 316–324.

Kameoka, S., Duque, P., and Konarska, M.M. (2004). p54(nrb) associates with the 5′ splice site within large transcription/splicing complexes. EMBO J. *23*, 1782–1791.

Kaneko, S., Rozenblatt-Rosen, O., Meyerson, M., and Manley, J.L. (2007). The multifunctional protein p54nrb/PSF recruits the exonuclease XRN2 to facilitate pre-mRNA 3′ processing and transcription termination. Genes Dev. *21*, 1779–1789.

Kapranov, P., Cheng, J., Dike, S., Nix, D.A., Duttagupta, R., Willingham, A.T., Stadler, P.F., Hertel, J., Hackermuller, J., Hofacker, I.L., et al. (2007). RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science *316*, 1484–1488.

Kashima, I., Yamashita, A., Izumi, N., Kataoka, N., Morishita, R., Hoshino, S., Ohno, M., Dreyfuss, G., and Ohno, S. (2006). Binding of a novel SMG-1-Upf1-eRF1-eRF3 complex (SURF) to the exon junction complex triggers Upf1 phosphorylation and nonsense-mediated mRNA decay. Genes Dev. *20*, 355–367.

Kim, M., Krogan, N.J., Vasiljeva, L., Rando, O.J., Nedea, E., Greenblatt, J.F., and Buratowski, S. (2004). The yeast Rat1 exonuclease promotes transcription termination by RNA polymerase II. Nature *432*, 517–522.

Kim, Y.K., and Kim, V.N. (2007). Processing of intronic microRNAs. EMBO J. *26*, 775–783.

Kohler, A., and Hurt, E. (2007). Exporting RNA from the nucleus to the cytoplasm. Natl. Rev. *8*, 761–773.

Komarnitsky, P., Cho, E.J., and Buratowski, S. (2000). Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev. *14*, 2452–2460.

Krishnamurthy, S., He, X., Reyes-Reyes, M., Moore, C., and Hampsey, M. (2004). Ssu72 Is an RNA polymerase II CTD phosphatase. Mol. Cell *14*, 387–394.

Kundu, S., Horn, P.J., and Peterson, C.L. (2007). SWI/SNF is required for transcriptional memory at the yeast GAL gene cluster. Genes Dev. *21*, 997–1004.

Lacadie, S.A., and Rosbash, M. (2005). Cotranscriptional spliceosome assembly dynamics and the role of U1 snRNA:5′ss base pairing in yeast. Mol. Cell *19*, 65–75.

Lacadie, S.A., Tardiff, D.F., Kadener, S., and Rosbash, M. (2006). In vivo commitment to yeast cotranscriptional splicing is sensitive to transcription elongation mutants. Genes Dev. *20*, 2055–2066.

Le Hir, H., and Andersen, G.R. (2008). Structural insights into the exon junction complex. Curr. Opin. Struct. Biol. *18*, 112–119.

Le Hir, H., and Seraphin, B. (2008). EJCs at the heart of translational control. Cell *133*, 213–216.

Le Hir, H., Izaurralde, E., Maquat, L.E., and Moore, M.J. (2000). The spliceosome deposits multiple proteins 20-24 nucleotides upstream of mRNA exon-exon junctions. EMBO J. *19*, 6860–6869.

Le Hir, H., Nott, A., and Moore, M.J. (2003). How introns influence and enhance eukaryotic gene expression. Trends Biochem. Sci. *28*, 215–220.

Lecuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., Hughes, T.R., Tomancak, P., and Krause, H.M. (2007). Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. Cell *131*, 174–187.

Lejeune, F., Ishigaki, Y., Li, X., and Maquat, L.E. (2002). The exon junction complex is detected on CBP80-bound but not eIF4E-bound mRNA in mammalian cells: dynamics of mRNP remodeling. EMBO J. *21*, 3536–3545.

Li, B., Carey, M., and Workman, J.L. (2007). The role of chromatin during transcription. Cell *128*, 707–719.

Li, X., and Manley, J.L. (2005). Inactivation of the SR protein splicing factor ASF/SF2 results in genomic instability. Cell *122*, 365–378.

Licatalosi, D.D., Geiger, G., Minet, M., Schroeder, S., Cilli, K., McNeil, J.B., and Bentley, D.L. (2002). Functional interaction of yeast pre-mRNA 3′ end processing factors with RNA polymerase II. Mol. Cell *9*, 1101–1111.

Lin, S., Coutinho-Mansfield, G., Wang, D., Pandit, S., and Fu, X.D. (2008). The splicing factor SC35 has an active role in transcriptional elongation. Nat. Struct. Mol. Biol. *15*, 819–826.

Longman, D., Johnstone, I.L., and Caceres, J.F. (2003). The Ref/Aly proteins are dispensable for mRNA export and development in Caenorhabditis elegans. RNA *9*, 881–891.

Luo, M.J., and Reed, R. (1999). Splicing is required for rapid and efficient mRNA export in metazoans. Proc. Natl. Acad. Sci. USA *96*, 14937–14942.

Ma, X.M., Yoon, S.O., Richardson, C.J., Julich, K., and Blenis, J. (2008). SKAR links pre-mRNA splicing to mTOR/S6K1-mediated enhanced translation efficiency of spliced mRNAs. Cell *133*, 303–313.

Mandel, C.R., Keneko, S., Zhang, H., Gebauer, D., Venthantham, V., Manley, J.L., and Tong, L. (2006). Polyadenylation factor CPSF-73 is the pre-mRNA 3′-end-processing endonuclease. Nature *444*, 953–956.

Manley, J.L. (2002). Nuclear coupling: RNA processing reaches back to transcription. Nat. Struct. Biol. *9*, 790–791.

Marcello, A., Lusic, M., Pegoraro, G., Pellegrini, V., Beltram, F., and Giacca, M. (2004). Nuclear organization and the control of HIV-1 transcription. Gene *326*, 1–11.

Masuda, S., Das, R., Cheng, H., Hurt, E., Dorman, N., and Reed, R. (2005). Recruitment of the human TREX complex to mRNA during splicing. Genes Dev. *19*, 1512–1517.

Masuyama, K., Taniguchi, I., Kataoka, N., and Ohno, M. (2004). RNA length defines RNA export pathway. Genes Dev. *18*, 2074–2085.

McGlincy, N.J., and Smith, C.W. (2008). Alternative splicing resulting in nonsense-mediated mRNA decay: what is the meaning of nonsense? Trends Biochem. Sci. *33*, 385–393.

Meinhart, A., Kamenski, T., Hoeppner, S., Baumli, S., and Cramer, P. (2005). A structural perspective of CTD function. Genes Dev. *19*, 1401–1415.

Merz, C., Urlaub, H., Will, C.L., and Luhrmann, R. (2007). Protein composition of human mRNPs spliced in vitro and differential requirements for mRNP protein recruitment. RNA *13*, 116–128.

Michlewski, G., Sanford, J.R., and Caceres, J.F. (2008). The splicing factor SF2/ASF regulates translation initiation by enhancing phosphorylation of 4E-BP1. Mol. Cell *30*, 179–189.

Moore, M.J. (2005). From birth to death: the complex lives of eukaryotic mRNAs. Science *309*, 1514–1518.

Morlando, M., Ballarino, M., Greco, P., Caffarelli, E., Dichtl, B., and Bozzoni, I. (2004). Coupling between snoRNP assembly and 3′ processing controls box C/D snoRNA biosynthesis in yeast. EMBO J. *23*, 2392–2401.

Morlando, M., Ballarino, M., Gromak, N., Pagano, F., Bozzoni, I., and Proudfoot, N.J. (2008). Primary microRNA transcripts are processed co-transcriptionally. Nat. Struct. Mol. Biol. *15*, 902–909.

Muse, G.W., Gilchrist, D.A., Nechaev, S., Shah, R., Parker, J.S., Grissom, S.F., Zeitlinger, J., and Adelman, K. (2007). RNA polymerase is poised for activation across the genome. Nat. Genet. *39*, 1507–1511.

Nagy, E., and Maquat, L.E. (1998). A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. Trends Biochem. Sci. *23*, 198–199.

Ng, H.H., Robert, F., Young, R.A., and Struhl, K. (2003). Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol. Cell *11*, 709–719.

Nguyen, V.T., Kiss, T., Michels, A.A., and Bensaude, O. (2001). 7SK small nuclear RNA binds to and inhibits the activity of CDK9/cyclin T complexes. Nature *414*, 322–325.

O'Sullivan, J.M., Tan-Wong, S.M., Morillon, A., Lee, B., Coles, J., Mellor, J., and Proudfoot, N.J. (2004). Gene loops juxtapose promoters and terminators in yeast. Nat. Genet. *36*, 1014–1018.

Orphanides, G., and Reinberg, D. (2002). A unified theory of gene expression. Cell *108*, 439–451.

Palacios, I.M. (2002). RNA processing: splicing and the cytoplasmic localisation of mRNA. Curr. Biol. *12*, R50–R52.

Palacios, I.M., Gatfield, D., St Johnston, D., and Izaurralde, E. (2004). An eIF4AIII-containing complex required for mRNA localization and nonsense-mediated mRNA decay. Nature *427*, 753–757.

Perkins, K.J., Lusic, M., Mitar, I., Giacca, M., and Proudfoot, N.J. (2008). Transcription-dependent gene looping of the HIV-1 provirus is dictated by recognition of pre-mRNA processing signals. Mol. Cell *29*, 56–68.

Peterlin, B.M., and Price, D.H. (2006). Controlling the elongation phase of transcription with P-TEFb. Mol. Cell *23*, 297–305.

Proudfoot, N. (2004). New perspectives on connecting messenger RNA 3′ end formation to transcription. Curr. Opin. Cell Biol. *16*, 272–278.

Pulak, R., and Anderson, P. (1993). mRNA surveillance by the Caenorhabditis elegans smg genes. Genes Dev. *7*, 1885–1897.

Rosner, A., and Rinkevich, B. (2007). The DDX3 subfamily of the DEAD box helicases: divergent roles as unveiled by studying different organisms and in vitro assays. Curr. Med. Chem. *14*, 2517–2525.

Rosonina, E., and Manley, J.L. (2005). From transcription to mRNA: PAF provides a new path. Mol. Cell *20*, 167–168.

Rougemaille, M., Dieppois, G., Kisseleva-Romanova, E., Gudipati, R.K., Lemoine, S., Blugeon, C., Boulay, J., Jensen, T.H., Stutz, F., Devaux, F., et al. (2008). THO/Sub2p functions to coordinate 3′-end processing with gene-nuclear pore association. Cell *135*, 308–321.

Saguez, C., Schmid, M., Olesen, J.R., Ghazy, M.A., Qu, X., Poulsen, M.B., Nasser, T., Moore, C., and Jensen, T.H. (2008). Nuclear mRNA surveillance in THO/sub2 mutants is triggered by inefficient polyadenylation. Mol. Cell *31*, 91–103.

Sanford, J.R., Gray, N.K., Beckmann, K., and Caceres, J.F. (2004). A novel role for shuttling SR proteins in mRNA translation. Genes Dev. *18*, 755–768.

Schroeder, S.C., Schwer, B., Shuman, S., and Bentley, D. (2000). Dynamic association of capping enzymes with transcribing RNA polymerase II. Genes Dev. *14*, 2435–2440.

Shen, H., Zheng, X., Shen, J., Zhang, Z., Zhao, R., and Green, M.R. (2008). Distinct activities of the DExD/H-box splicing factor hUAP56 facilitate stepwise assembly of the spliceosome. Genes Dev. *22*, 1796–1803.

Shibuya, T., Tange, T.O., Sonenberg, N., and Moore, M.J. (2004). eIF4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. Nat. Struct. Mol. Biol. *11*, 346–351.

Shilatifard, A. (2006). Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. Annu. Rev. Biochem. *75*, 243–269.

Shuman, S. (2001). Structure, mechanism, and evolution of the mRNA capping apparatus. Prog. Nucleic Acid Res. Mol. Biol. *66*, 1–40.

Sims, R.J., 3rd, Millhouse, S., Chen, C.F., Lewis, B.A., Erdjument-Bromage, H., Tempst, P., Manley, J.L., and Reinberg, D. (2007). Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing. Mol. Cell *28*, 665–676.

Singh, B.N., and Hampsey, M. (2007). A transcription-independent role for TFIIB in gene looping. Mol. Cell *27*, 806–816.

Schmid, M., and Jensen, T.H. (2008). The exosome: a multipurpose RNA-decay machine. Trends Biochem. Sci. *33*, 501–510.

Stalder, L., and Muhlemann, O. (2008). The meaning of nonsense. Trends Cell Biol. *18*, 315–321.

Steinmetz, E.J., Conrad, N.K., Brow, D.A., and Corden, J.L. (2001). RNA-binding protein Nrd1 directs poly(A)-independent 3′-end formation of RNA polymerase II transcripts. Nature *413*, 327–331.

Tange, T.O., Nott, A., and Moore, M.J. (2004). The ever-increasing complexities of the exon junction complex. Curr. Opin. Cell Biol. *16*, 279–284.

Thermann, R., Neu-Yilik, G., Deters, A., Frede, U., Wehr, K., Hagemeier, C., Hentze, M.W., and Kulozik, A.E. (1998). Binary specification of nonsense codons by splicing and cytoplasmic translation. EMBO J. *17*, 3484–3494.

Valencia, P., Dias, A.P., and Reed, R. (2008). Splicing promotes rapid and efficient mRNA export in mammalian cells. Proc. Natl. Acad. Sci. USA *105*, 3386–3391.

Vasiljeva, L., and Buratowski, S. (2006). Nrd1 interacts with the nuclear exosome for 3′ processing of RNA polymerase II transcripts. Mol. Cell *21*, 239–248.

Vasiljeva, L., Kim, M., Mutschler, H., Buratowski, S., and Meinhart, A. (2008). The Nrd1-Nab3-Sen1 termination complex interacts with the Ser5-phosphorylated RNA polymerase II C-terminal domain. Nat. Struct. Mol. Biol. *15*, 795–804.

West, S., Gromak, N., and Proudfoot, N.J. (2004). Human 5′–> 3′ exonuclease Xrn2 promotes transcription termination at co-transcriptional cleavage sites. Nature *432*, 522–525.

West, S., Proudfoot, N.J., and Dye, M.J. (2008). Molecular dissection of mammalian RNA polymerase II transcriptional termination. Mol. Cell *29*, 600–610.

Wu, W.H., Pinto, I., Chen, B.S., and Hampsey, M. (1999). Mutational analysis of yeast TFIIB. A functional relationship between Ssu72 and Sub1/Tsp1 defined by allele-specific interactions with TFIIB. Genetics *153*, 643–652.

Yoh, S.M., Cho, H., Pickle, L., Evans, R.M., and Jones, K.A. (2007). The Spt6 SH2 domain binds Ser2-P RNAPII to direct Iws1-dependent mRNA splicing and export. Genes Dev. *21*, 160–174.

Zacharioudakis, I., Gligoris, T., and Tzamarias, D. (2007). A yeast catabolic enzyme controls transcriptional memory. Curr. Biol. *17*, 2041–2046.

Zhang, J., Sun, X., Qian, Y., LaDuca, J.P., and Maquat, L.E. (1998). At least one intron is required for the nonsense-mediated decay of triosephosphate isomerase mRNA: a possible link between nuclear splicing and cytoplasmic translation. Mol. Cell. Biol. *18*, 5272–5283.

Zhang, Z., and Krainer, A.R. (2004). Involvement of SR proteins in mRNA surveillance. Mol. Cell *16*, 597–607.

Zhang, Z., and Krainer, A.R. (2007). Splicing remodels messenger ribonucleoprotein architecture via eIF4A3-dependent and -independent recruitment of exon junction complex components. Proc. Natl. Acad. Sci. USA *104*, 11574–11579.