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Early Formation of mRNP: License for Export or Quality Control?

Review

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Eukaryotic mRNA is processed by enzymes and packaged with proteins within nuclei to generate functional messenger ribonucleoprotein (mRNP) particles. Processing and packaging factors can interact with mRNA cotranscriptionally to form an early mRNP. Erroneous mRNP formation leads to nuclear retention and degradation of the mRNA. It therefore appears that one function of cotranscriptional mRNP assembly is to discard aberrant mRNPs early in their biogenesis. Cotranscriptional mRNP assembly may also enable the transcription machinery to respond to improper mRNP formation.

Introduction

Pre-mRNA undergoes nuclear processing to become mature mRNA, which is then exported to the cytoplasm and translated. These modifications generate a translatable open reading frame and protect the transcript from premature degradation. It has been established that pre-mRNA processing steps are interconnected, with each other and with transcription (for recent reviews see Neugebauer, 2002; Proudfoot et al., 2002; Reed and Hurt, 2002). The basis for such coupling is due in part to the coordination of processing with transcription, through physical interactions of processing factors with the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (RNAPII, Figure 1). RNA is also packaged cotranscriptionally into mRNP, and mRNA export factors have been shown to load onto nascent transcripts (Figure 1). In one view, coupling between the transcription machinery and components of the processing-transport systems is required for the nascent, step-wise construction of an export competent mRNP. mRNA export would therefore require cotranscriptional mRNP assembly. However, mRNA processing is not always coupled to transcription, and transcription by RNAPII is not essential for many aspects of mRNP assembly and export. Cotranscriptional mRNP assembly may therefore also serve to allow early monitoring of mRNP quality. This is because failure to form proper mRNP leads to retention by a transcription site-associated surveillance system and ultimately to mRNA degradation.

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mRNA Processing and RNAPII: Yeast versus Higher Eukaryotes

Early pre-mRNA processing events include addition of a 5′ methylated guanosine cap structure, splicing to remove intronic sequences, and 3′ end formation, which results in a stretch of 3′ adenosine residues (the poly(A)-tail). These covalent modifications are essential for proper mRNA function, and all three occur in vitro without transcription. In vivo, however, transcription by RNAPII is important for mRNA processing. This appears less true, or less strictly true, in yeast than in metazoans. The word "appears" was carefully chosen, as few scientists have successfully bet on a major difference in gene expression between yeast and metazoans. With few exceptions, differences between these two systems turned out to be more apparent than real. However, the genome projects have shown conclusively that some metazoan RNA processing proteins are not present in *S. cerevisiae*. There is no *S. cerevisiae* ortholog of U2AF35, the small subunit of the splicing factor U2AF. In addition, there are apparently no yeast splicing orthologs of metazoan SR proteins, which validates described differences in splicing complex formation between these organisms. For example, the prominent role of ASF/SF2 in early splicing complex formation in mammals appears to have no equivalent in yeast.

Capping

This is the best understood mRNA processing event and is essentially without controversy in both systems. Shortly after transcription initiation, a 7-methylguanosine (7mG) cap is added to the 5′ end of the nascent transcript. In both yeast and metazoans, capping is clearly coupled to transcription by RNAPII. To date, there are only two ways to generate a capped transcript in vivo: transcription by RNAPII or transcription by viral RNA polymerases which bypass this host cell requirement. Capping enzyme activity in vivo is restricted to sites of RNAPII transcription through physical associations with the CTD and with RNAPII-associated transcription factors (Cho et al., 1997; Lindstrom et al., 2003; McCracken et al., 1997a). The capping enzymes are present toward the 5′ end of genes, as determined by chromatin immunoprecipitation (ChIP) assays, and nuclear cap binding proteins recognize the 7mG cap structure cotranscriptionally (Komarnitsky et al., 2000; Schroeder et al., 2000; Visa et al., 1996). Capping therefore occurs cotranscriptionally, and a restriction to RNAPII transcripts is presumably mediated by RNAPII directly: recombinant CTD stimulates in vitro capping, and RNAPII allosterically regulates capping enzyme activity (Cho et al., 1998; Ho and Shuman, 1999; Wen and Shatkin, 1999). The only known difference between yeast and mammalian systems is that the mammalian capping triphosphatase and guanylyltransferase activi-

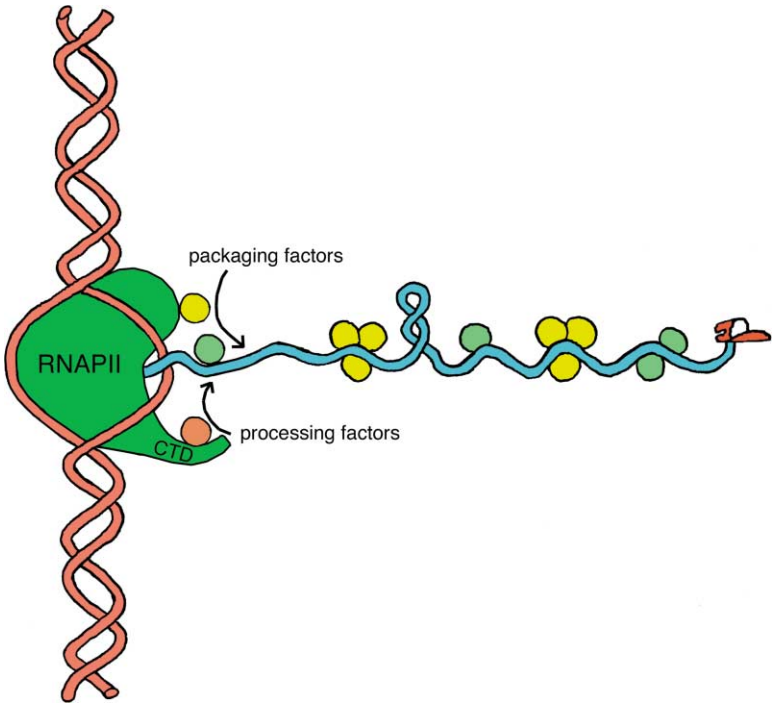

Figure 1. Cotranscriptional mRNP Formation

The nascent transcript emerging from RNAPII is processed by enzymes and packaged with proteins to form a mature mRNP. In vivo, these events are modulated by RNAPII, the extent to which differs between experimental systems (see text for details).

ties are a single polypeptide, whereas in yeast they are separate proteins.

Splicing

Higher eukaryotic splicing can occur cotranscriptionally. This has been known for the last 15 years, starting with the pioneering electron microscopy studies of Beyer and colleagues on *Drosophila* chromosomes; they showed partially spliced transcripts still attached to transcribing RNAPII (Osheim et al., 1985). These observations were confirmed in other experimental systems, including the colocalization of splicing factors with transcribing RNAPII in HeLa nuclei (Bauren and Wieslander, 1994; Misteli and Spector, 1999). Splicing efficiency in vivo is dependent on the integrity of RNAPII, and the CTD stimulates this process independently on its involvement in other mRNA processing reactions (Du and Warren, 1997; Fong and Bentley, 2001; McCracken et al., 1997b). As a consequence, alternative splicing is affected by promoter choice and the elongating rate of RNAPII (Cramer et al., 1999; Kadener et al., 2002). Consistent with a CTD requirement, intron-containing transcripts produced by RNAP I or RNAPIII in higher eukaryotes are inefficiently spliced (Sisodia et al., 1987; Smale and Tjian, 1985).

Not all mammalian splicing is cotranscriptional, as there are transcripts known to splice posttranscriptionally (Neugebauer, 2002). This fits well with *Xenopus* oocyte injection experiments, in which RNAs are generally efficiently spliced in the absence of transcription (Green et al., 1983). It also fits with the fact that in vitro splicing does not require transcription although it is stimulated by the addition of phosphorylated RNAPII (Hirose et al., 1999). In this posttranscriptional view, the negative results using RNAPI and RNAPIII in mammalian systems require an alternative explanation, such as a role for chromatin structure in splicing or a deleterious effect of the non-RNAPII polymerase. The CTD or RNAPII may also contribute indirectly to splicing factor recruitment, through a direct effect on capping or on a splicing-relevant aspect of transcription such as polymerase processivity (Kadener et al., 2002). Importantly, splicing factor recruitment as defined immunohistochemically does not require cotranscriptional splicing; high concentrations of splicing factors localize near active genes without being precisely coincident with nascent transcription complexes (Johnson et al., 2000; Jolly et al., 1999; Sacco-Bubulya and Spector, 2002).

The yeast system appears to differ significantly from higher eukaryotes, as cotranscriptional splicing has yet to be reported. Splicing of nested yeast introns showed that splice site recognition can occur after a first splicing event and therefore separate from transcription (Lopez and Seraphin, 2000). Furthermore, the relatively inefficient splicing of T7 RNA polymerase (T7RNAP)-directed transcripts in yeast is almost entirely explained by the lack of a cap (Dower and Rosbash, 2002). Thus, the stimulatory role of RNAPII in yeast splicing may work indirectly through the need for efficient capping.

3′ End Formation

RNA polyadenylation signals direct cleavage and polyadenylation at the 3′ end of the transcript (Zhao et al., 1999). The two reactions are tightly coupled and result in poly(A) tail lengths of 70–90 nucleotides in yeast and 200–300 nucleotides in mammals. The most extraordinary feature of the cellular cleavage and polyadenylation machinery is its complexity: many factors are required for what could in theory be carried out by a single endonuclease and poly(A) polymerase (PAP) (Proudfoot and O'Sullivan, 2002). Higher eukaryotic polyadenylation signals normally go unrecognized unless the transcript is made by RNAPII (Gunnery and Mathews, 1995; Sisodia et al., 1987). This is generally explained by physical asso-

ciation of the CTD with the 3′ end formation machinery (Proudfoot et al., 2002). However, there are some reports that this process can occur posttranscriptionally and even independently of RNAPII (Fodor et al., 2000; Lewis and Manley, 1986; Osheim et al., 1999).

The contribution of RNAPII to yeast 3′ end formation—like yeast splicing—is more subtle. Non-RNAPII transcripts are efficiently cleaved and polyadenylated (Dower and Rosbash, 2002; Lo et al., 1998; McNeil et al., 1998). However, some 3′ end formation defects are observed for these transcripts as well as for those generated by yeast RNAPII lacking a CTD (Licatalosi et al., 2002). Moreover, antibodies against yeast 3′ end formation factors can immunoprecipitate chromatin, demonstrating that they are present when the transcript is still nascent (Komarnitsky et al., 2000; Licatalosi et al., 2002). Yeast RNAPII is therefore dispensable for 3′ end formation but may play a role in this process.

The CTD

The CTD consists of heptad repeats of the consensus sequence YSPTSPS, 26 repeats in yeast and 52 in mammals. It is essential for viability, and truncations in yeast of approximately half the repeats causes conditional lethality (Nonet et al., 1987; West and Corden, 1995). Whether CTD interactions with mRNA processing factors are essential is not known. Surprisingly, none of the yeast genetic screens done with the CTD has pointed to a role in processing; i.e., no processing factors have turned up in suppressor or enhancer screens, which have only yielded factors with roles in transcriptional activation (Nonet and Young, 1989; Yuryev and Corden, 1996).

An insightful distinction between the yeast and mammalian CTD has arisen from two recent results. Fong and Bentley showed that heptads 1–26 of the mammalian CTD support only capping, whereas heptads 27–52 are required to maintain coupling of capping, splicing, and 3′ end processing (Fong and Bentley, 2001). Heptads 1–26 are more similar to the yeast CTD than to heptads 27–52: the yeast CTD contains 19 repeats that perfectly match the YSPTSPS consensus, and the mammalian CTD has 21, 19 of which are in heptads 1–26. In another report, Ryan et al. used CTD-mediated stimulation of an in vitro 3′ end formation assay to demonstrate that more than 26 repeats are required for full activity (Ryan et al., 2002). These results are consistent with the more prominent role of the mammalian CTD in splicing and 3′ end formation as compared to the yeast CTD. The complexity of mammalian transcripts may require a more sophisticated level of coordination between processing steps. Not all eukaryotes have a CTD, and it has been suggested that the evolutionary emergence of the CTD was a critical step in affording this greater control over gene expression (Stiller and Hall, 2002). Interestingly, eukaryotes lacking a CTD have few intron-containing genes. Yeast has relatively few (and simple) introns and may have an intermediate degree of coupling between transcription and splicing. Indeed, there is evidence that yeast splicing factors can interact directly with RNAPII (Morris and Greenleaf, 2000). This modest degree of coupling may extend to yeast 3′ end formation, as yeast 3′ end formation factors have also been shown to interact with the CTD (Barilla et al., 2001; Dichtl et al., 2002b).

Effects of mRNA Processing on Transcription

Cotranscriptional mRNA processing is a dynamic phenomenon. Several polymerases may be simultaneously engaged in transcribing a single gene, which necessitates efficiency or coordination of the transcription/processing events to avoid interference or stalling. As discussed above, mRNA processing is in some cases aided by RNAPII, in part through interactions with processing factors. However, recent results indicate that these interactions may be as important for regulating RNAPII activity as for RNA processing.

Capping occurs when the nascent transcript is roughly 20 nucleotides in length and is inefficient if transcription elongation occurs unimpeded (Chiu et al., 2001). To provide a temporal window for capping execution, a transcriptional delay is mediated in vertebrates through binding of the negative transcriptional regulator DSIF (hSpt5/hSpt4) to RNAPII (Chiu et al., 2002; Pei and Shuman, 2002). Transcription continues when the heterodimeric pTEF-b kinase phosphorylates hSpt5 and the CTD. Recent work in *S. pombe* showed that the capping triphosphatase interacts with Cdk9, an ortholog of a human subunit of pTEF-b, suggesting a contribution to release from transcriptional arrest by this capping enzyme (Pei et al., 2003). In mammalian cells, the HIV-1 transcriptional *trans*-activator Tat may also exercise a positive effect on elongation by targeting capping activity to arrested viral transcription complexes (Chiu et al., 2002). Intriguingly, the capping apparatus in *S. cerevisiae* can also repress RNAPII transcription. Results from in vitro as well as in vivo transcription assays are consistent with an inhibitory role of the *S. cerevisiae* triphosphatase Cet1p on transcription reinitiation (Myers et al., 2002). Thus, the capping complex appears to promote transcriptional elongation of the freshly capped transcript, while delaying synthesis of the next one. It has been suggested that this phenomenon could constitute a checkpoint to minimize uncapped mRNA (Manley, 2002).

The evidence for an influence of splicing on transcription is paradoxically stronger than vice versa. Recent work from two different laboratories indicates that interactions between splicing and transcription factors promote both transcriptional initiation and elongation in vitro (Fong and Zhou, 2001; Kwek et al., 2002). In the first example, a highly purified preparation of the transcriptional initiation factor TFIIH contained stoichiometric amounts of U1snRNA, and this complex was more potent in both initiation and reinitiation than TFIIH alone (Kwek et al., 2002). In addition, a promoter proximal 5′-splice site was required for U1snRNA-dependent stimulation of reinitiation, suggesting a requirement for splice site recognition. In another report, the authors focused on a transcriptional elongation activity associated with p-TEFb (Fong and Zhou, 2001). This activity was identified as TAT-SF1, a general transcription elongation factor. TAT-SF1 is also a putative ortholog of the *S. cerevisiae* splicing factor Cus2p, which is associated with U2 snRNP. Interestingly, TAT-SF1 was found in association with all spliceosomal snRNPs, and the complex was active both in splicing and transcription elongation. Activity was notably stimulated by the presence of splicing signals. These results are corroborated by a recent analysis demonstrating that introns increase the

density of transcriptionally active RNAPII in both yeast and higher eukaryotes (Furger et al., 2002).

The contribution of 3′ end processing to transcriptional termination is well established (Proudfoot et al., 2002). Efficient termination in higher eukaryotes is dependent on the presence of a functional poly(A) signal. However, transcription termination factors as well as sequences downstream of the polyadenylation site are also required. Although such downstream sequences are ill defined, they may provide pause sites for RNAPII. According to a recently proposed model, transcribed sequences downstream of the polyadenylation site might also provide for cotranscriptional transcript cleavage (CoTC; Dye and Proudfoot, 2001). CoTC refers to cleavage events preceding the final cut, which defines the site for poly(A) addition and occurs independently of normal 3′ end processing. In human β-globin pre-mRNA, sequence tracts located 900–1600 bp downstream of the polyadenylation signal are required for transcriptional termination and trigger CoTC (Dye and Proudfoot, 2001). It has been suggested that RNAPII termination occurs in two steps. First, interactions between RNAPII-bound polyadenylation factors and the polyadenylation signal within nascent RNA would cause continued transcription to loop out downstream RNA sequences. The primary termination event would then be CoTC, causing RNAPII to stop transcription. Second, cleavage at the poly(A) site would release RNAPII from the RNA and from chromatin. Whether this interesting model is generally valid remains to be seen.

Transfer of factors from RNAPII to the nascent polyadenylation signal may affect RNAPII processivity and trigger the termination process. *S. cerevisiae* 3′ end processing factors are recruited to RNAPII near the promoter and remain associated until the 3′ end of the gene (Licatalosi et al., 2002). Furthermore, the Ssu72p and Sub1p proteins, originally implicated in transcription, have recently been shown to be associated with the 3′ end processing machinery (He et al., 2003; Dichtl et al., 2002a). Association of such factors with both RNAPII and 3′-processing signals positions them as potential modulators of RNAPII activity.

mRNP Export and Retention

Cells have nonoverlapping RNA export pathways, which was first established by microinjection experiments in *Xenopus* oocytes. RNA of different classes (i.e., tRNA, UsnRNA, and mRNA) competes with the export of a radiolabeled RNA of the same class but not of another class (Jarmolowski et al., 1994). It is interesting to note (in hindsight of course) that these pioneering injection studies avoided three contemporary issues in mRNA export: (1) retention of aberrant mRNPs at or near their site of synthesis, (2) connections between export and transcription, and (3) a contribution of splicing to mRNA export.

Transcription-Site Release of mRNP

Microinjected RNAs bypass transcription and are still exported in the absence of a cap, splicing, or a poly(A) tail. In yeast, the minor role of capping is illustrated by uncapped T7RNAP-derived transcripts, which are substrates for nuclear export (Dower and Rosbash, 2002). RNAPII transcripts in yeast mutants of the guanyltransferase capping enzyme are also not retained in the nucleus (Fresco and Buratowski, 1996). In contrast, both splicing and 3′ end formation have a measurable influence on the mRNA export of in vivo transcribed RNAs. Splicing may only have a stimulatory role in mRNA export, but correct 3′ end formation appears required.

Evidence linking proper 3′ end formation to mRNA export has existed for some time in both yeast and higher eukaryotes (Eckner et al., 1991; Huang and Carmichael, 1996; Long et al., 1995). Using transcript-specific RNA fluorescent in situ hybridization (RNA-FISH) analysis, it was observed that human β-globin RNA defective for 3′ end cleavage colocalized with the template gene locus in a stably transfected murine erythroleukemia cell system (Custodio et al., 1999). Yeast transcripts accumulate in intranuclear foci suggested to be transcription sites, whenever 3′ end formation is affected in *trans* or in *cis* (Dower and Rosbash, 2002; Hilleren et al., 2001; Libri et al., 2002). Surprisingly, RNA-FISH signals can also stem from nonnascent RNA, as adenylated yeast heat shock RNAs (hs-RNAs) accumulate at such sites under conditions where export is blocked (Jensen et al., 2001b). These observations suggest that a transcription-site release step exists, even for fully processed mRNA (Figure 2). It should be noted that the term "transcription site" is presently ill defined. The molecular constituents of such sites are unknown, and the exact distance between the gene and the sequestered RNA has not yet been evaluated with high-resolution techniques. Nonetheless, the yeast results raise the possibility that some mammalian mRNA signals at transcription sites may result from fully processed RNAs.

Cotranscriptional Loading of Export Factors

Similar to mRNA processing factors, proteins involved in RNA-packaging and export are associated with transcriptionally active genes. Work primarily from yeast indicates that the nascent RNA is contacted by such factors, which function at the interface between transcription and mRNP formation. A prominent example is the RNA binding nucleo-cytoplasmic shuttling protein Npl3p, which interacts physically with RNAPII and genetically with the TATA box binding protein (Lei et al., 2001). In ChIP assays, Npl3p is present along the entire length of the gene. Although the exact function of Npl3p is unknown, it may contribute to some aspect of mRNP integrity. The yeast THO complex composed of four components (Hpr1p, Mft1p, Tho2p and Thp1p) has received recent attention. These proteins were first described as transcription elongation factors (Chavez and Aguilera, 1997; Chavez et al., 2000). Like Npl3p, the THO complex interacts both with RNAPII as well as with nascent RNA and is present at sites of active transcription (Chang et al., 1999; Strasser et al., 2002; Zenklusen et al., 2002). Interestingly, the yeast mRNA export factors Sub2p and Yra1p are stoichiometrically associated with the THO components in the newly coined TREX (TRanscription/EXport) complex (Strasser et al., 2002). Deletion or mutation of any of the TREX components results in rapid nuclear accumulation of polyadenylated mRNA as well as sequestration of hs-RNAs at their sites of transcription (Figure 2A, Jensen et al., 2001a; Libri et al., 2002; Strasser et al., 2002; Zenklusen et al., 2002). The THO complex is proposed to bring Sub2p and Yra1p to the nascent mRNP, and biochemical experiments

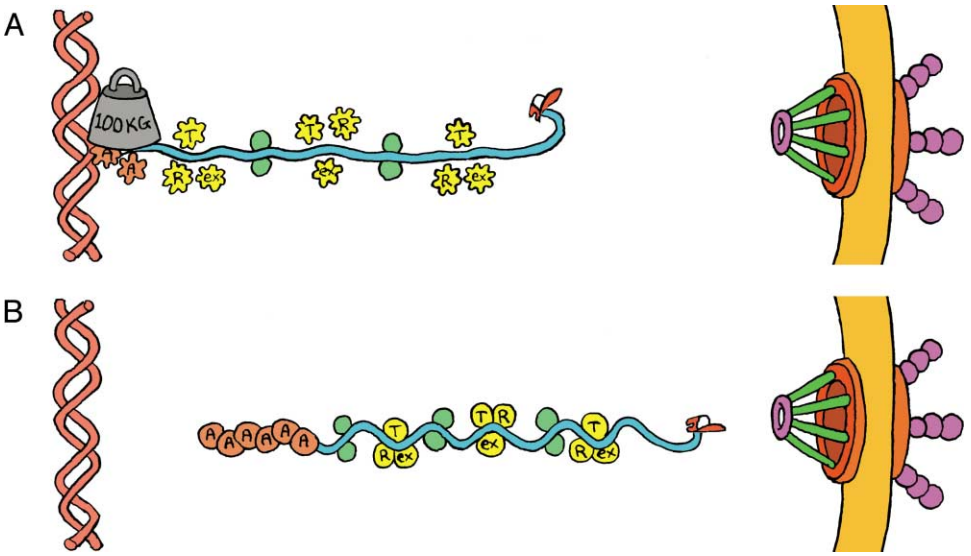

Figure 2. Release of an mRNP from Its Transcription Site

(A) Abnormalities in *cis* or in *trans* leads to retention of the mRNP near its site of transcription.

(B) An early mRNP has been loaded with factors important for its functional progress. Features required for transcription-site release of the mRNP are denoted: the TREX complex (yellow), a poly(A) tail with a correct length bound by poly(A) binding proteins (brown), and additional factors important for mRNP integrity (green).

suggest that Yra1p serves as the universal adaptor protein between all mRNAs and the heterodimeric export receptor Mex67p/Mtr2p (Strasser and Hurt, 2001). The connection to RNAPII has the attractive feature that it explains how Yra1p would be deposited on all mRNAs independent of sequence or structure. However, a recent yeast microarray study showed that mRNPs containing Mex67p or Yra1p shared surprisingly few mRNAs (Hieronymus and Silver, 2003). Although a confirmation of this observation by a different experimental strategy is required, the data question the model, at least in its simplest form. Indeed, RNAi inactivation experiments in *Drosophila* cells indicate an essential mRNA export role of UAP56 (the metazoan ortholog of Sub2p) but not for Aly/REF (the metazoan ortholog of Yra1p), suggesting the existence of alternative recruitment modes and adaptors for mRNP transport (Gatfield and Izaurralde, 2002; Huang et al., 2003).

Given the importance of 3′ end formation for transcription-site release, it is prudent to consider in more detail the role of this process in mRNP export. Poly(A) tail length is carefully regulated, and it is tempting to speculate that it reflects the requirement of a defined number for proteins decorating the tail. In mammals, the nascent oligo(A) tail is bound by the nuclear PABPN1 protein, which regulates the extent of polyadenylation (Wahle and Ruegsegger, 1999). A PABPN1 ortholog does not exist in yeast, and the poly(A) binding proteins Pab1p and Nab2p have been suggested to regulate tail length (Brown and Sachs, 1998; Hector et al., 2002). Furthermore, hyperadenylated RNA species accumulate upon Nab2p depletion, with a concomitant block in mRNA export. However, hyperadenylation per se does not seem to trigger nuclear retention, as the mRNA export defect caused by Nab2p depletion is alleviated by Pab1p overexpression without shortening of the extended poly(A) tail. These observations rather suggest that nuclear retention is caused by the absence of the poly(A) binding factors Nab2p or Pab1p (Figure 2A), which then leads independently to excessive poly(A) addition.

Despite recent progress, much still needs to be learned about mRNP assembly, release, and export. For example, no experiment to date has addressed whether transcription-site release reflects export competence or an escape from active retention. Given the yeast literature on retention of aberrant mRNPs by the nuclear exosome (see below), we favor the latter possibility.

Splicing and mRNA Export

Splicing is not required for mRNA export, as evidenced from the standard practice of using cDNA expression constructs in higher eukaryotic systems. Nonetheless, a contribution of splicing to export is now well accepted. In higher eukaryotes, the Yra1p and Sub2p orthologs (Aly/REF and UAP56) are deposited as part of the exon-junction complex (EJC) on mRNA as a consequence of splicing (Gatfield et al., 2001; Le Hir et al., 2000). Indeed, EJC recruitment correlates with mRNA export ability in oocyte injection experiments (Le Hir et al., 2001). This positive effect may only be detectable on relatively short mRNAs, probably because of multiple low-affinity binding sites for transport factors on longer RNAs, which circumvent the need for splicing dependent recruitment (Rodrigues et al., 2001). As human (and presumably also *Xenopus*) counterparts of the yeast TREX complex components exist, these RNAs are also likely to acquire Aly/REF and UAP56 via this route (Strasser et al., 2002). Therefore, the EJC probably has a stimulatory effect on mRNA export, but its major role is likely exercised on other processes. Consistent with this notion, artificial insertion of an intron into a mammalian reporter construct had a greater impact on mRNA abundance and

translation than on nuclear-cytoplasmic distribution (Nott et al., 2003).

Despite the paucity of intron-containing genes in yeast, their transcripts are highly abundant and therefore constitute a significant fraction of cellular mRNAs. It is not clear whether an EJC exists in yeast. Although it is an attractive way for some mRNPs to acquire Yra1p, intron-containing RNAs did not constitute a separate group of Yra1p substrates in a yeast micro-array study (Hieronymus and Silver, 2003).

Cotranscriptional mRNP Surveillance

The term mRNA surveillance is usually associated with the nonsense-mediated decay (NMD) of transcripts harboring premature termination codons. However, recent studies in yeast indicate that a surveillance system monitors mRNP for other errors. In contrast to yeast NMD, the degradation leg of this system takes place in the nucleus, is closely connected to the transcription process and involves the RNA exosome, a complex of 3′ to 5′ exonucleases. Furthermore, the exosome not only functions in RNA degradation but also participates in the mRNA selection process.

Originally shown to process rRNAs, snoRNAs, and snRNAs in the nucleolus, this highly conserved multisubunit assembly also participates in cytoplasmic mRNA degradation in both yeast and mammalian cells (Butler, 2002; Jacobs Anderson and Parker, 1998; Mitchell et al., 1997; van Hoof et al., 2002; van Hoof and Parker, 2002). It was recently shown that the exosome also degrades nuclear mRNAs (Bousquet-Antonelli et al., 2000; Libri et al., 2002; Torchet et al., 2002; Zenklusen et al., 2002). In addition to the ten core subunits of the exosome, full nuclear activity requires an eleventh component, Rrp6p (PM-Scl 100KD in humans). By two criteria, Rrp6p is a unique constituent of the nuclear exosome: (1) it localizes strictly to the nucleus/nucleolus in both yeast and mammalian cells, and (2) yeast strains deleted for Rrp6p have defects only in nuclear exosome-mediated RNA processing events (Butler, 2002).

Evidence that the exosome participates in additional nuclear mRNA metabolic processes comes from a number of studies. The Butler laboratory identified two *rrp6* mutant alleles in a genetic screen for growth suppressors of the *pap1-1* ts-allele of the yeast poly(A) polymerase (Briggs et al., 1998). Rrp6p was subsequently shown to interact genetically and physically with Pap1p as well as with Npl3p, suggesting a link between the nuclear exosome and mRNP formation (Burkard and Butler, 2000). These results were substantiated by the observation that deletion of *RRP6* in the *pap1-1* strain background leads to release of unadenylated *SSA4* RNA, which is otherwise retained at its site of transcription in the *pap1-1* mutant strain (Hilleren et al., 2001). *SSA4* transcript release was not accompanied by major changes in *SSA4* RNA stability but rather by partial restoration of Ssa4p protein synthesis, suggesting that the unadenylated RNAs proceed normally to cytoplasmic polyribosomes upon removal of *RRP6*. All other known cases of transcription-site retention in yeast also require Rrp6p; i.e., aberrant mRNAs (or mRNPs) are not retained in an *RRP6* deletion background. These observations have led to a model in which Rrp6p, and possibly the entire nuclear exosome, surveys mRNPs, retaining and ultimately degrading aberrant transcripts.

Support for this model comes from *Drosophila*, where antibodies against exosome components stain transcriptionally active loci on polytene chromosomes (Andrulis et al., 2002). Recruitment of the exosome in this system may be mediated by the transcriptional elongation factors dSpt5 and dSpt6. These proteins associate not only with the large subunit of RNAP II but also with stoichiometric amounts of nine *Drosophila* orthologs of the yeast exosome. ChIP experiments for both dSpt5 and exosome components yield signals along the entire gene, suggesting that these factors become associated with RNAPII early in transcription. As Spt5 and Spt6 have established roles in transcriptional elongation in both yeast and humans, this suggests that the exosome-transcription link also exists in yeast.

Two lines of evidence indicate that the exosome degrades mRNA at transcription sites. First, dSpt6 immunoprecipitates possess exonucleolytic activity, demonstrating that the exosome is active while complexed with a transcriptional elongation factor (Andrulis et al., 2002). Second, the 3′ end truncated mRNAs observed in yeast strains mutated for TREX components are Rrp6p dependent (Libri et al., 2002; Zenklusen et al., 2002). Deletion of *RRP6* in a TREX mutant background not only restores proper levels of 3′ ends but also leads to transcript release from transcription-site foci (Libri et al., 2002). This suggests an explanation for transcript instability in TREX mutants, namely, improper mRNPs are retained with subsequent 3′–5′ degradation of the RNA.

As discussed above, some processing events do not always require RNAPII, and mRNA export factors are functional when loaded posttranscriptionally. It is therefore unlikely that cotranscriptional mRNP formation functions solely for nuclear export. Cotranscriptional loading may be more important for quality control purposes, to prevent improper mRNPs from progressing to the cytoplasm. What then defines a proper mRNP? Although we have no idea, the fact that the exosome is recruited to the 5′ end of transcribing genes suggests that the quality of very early mRNP events is monitored. Since the elongation factor Spt5 is involved in early transcriptional arrest, perhaps the exosome is even recruited to deal with unsuccessful capping events. However, early loading might not equal early action, as exemplified by the early recruitment of some 3′ end processing factors in both mammals and yeast.

Unspliced yeast pre-mRNAs are also substrates for the nuclear exosome, and nuclear degradation has been shown to compete with inefficient splicing (Bousquet-Antonelli et al., 2000). Whether this process occurs in transcription-site foci remains an open question and should be resolved by localizing the appropriate transcript substrates. However, the incomplete or uncertain coupling of yeast splicing to transcription indicates that degradation does not always take place close to the site of transcription. Indeed, a second mRNA quality checkpoint has been suggested to take place at the nuclear periphery (Green et al., 2003).

Most observations to date link the exosome to monitoring of proper 3′ end formation. Rrp6p-dependent transcription-site retention in yeast extends to hyperadenylated transcripts (in conventional mRNA export mu-

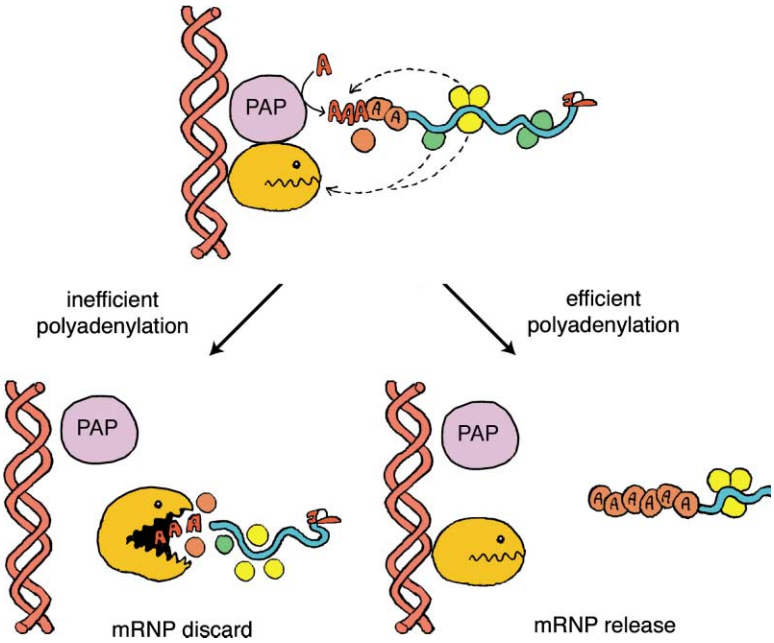

Figure 3. Current Model for mRNP Surveillance at Transcription Sites

The mRNP is retained close to its site of transcription by an exosome-dependent mechanism. This retention could be wrought by interactions between components of the exosome and the mRNP (indicated by lower dashed lines). Poly(A)-addition by PAP creates a potential substrate for the exosome (and perhaps additional nucleolytic enzymes). The efficiency of polyadenylation and/or addition of poly(A) binding proteins to the poly(A) tail could be affected by mRNP factors (indicated by the upper dashed line). Failure to create a proper tail leads to mRNA decay (lower left). Successful tail formation, on the other hand, leads to mRNP release (lower right).

tant strains), unadenylated transcripts (in the *pap1-1* strain), 3′ end extended transcripts (in 3′ end formation/transcription termination mutants), and to mRNAs with a free 3′ end generated by a self-cleaving hammerhead ribozyme (Hilleren et al., 2001; Jensen et al., 2001b; Libri et al., 2002). A mechanistic model must account for all these substrates, especially the fact that mRNA with fully processed 3′ ends can be retained. This suggests that exosome retention is not mediated solely by RNAPII and that mRNP components also physically interact with the exosome. Indeed, this is the case with unstable mRNAs containing AU-rich instability elements (AREs). In this example, cellular ARE binding factors or the AU-rich sequence itself recruits the cytoplasmic exosome (Chen et al., 2001; Mukherjee et al., 2002). Interestingly, early mRNP components (Yra1p and Npl3p) also interact with members of the nuclear exosome (Rrp45p and Rrp6p) (Burkard and Butler, 2000; Zenklusen et al., 2002). In addition, the exosome-interacting protein Spt6p has a reported interaction with histone H3, providing a mechanistic explanation for exosome tethering to chromatin (Bortvin and Winston, 1996). Some mRNP remodeling or conformational change would then sever the exosome-mRNP connection (or the exosome-chromatin connection) and release mRNP from the transcription site. Aberrant mRNPs might delay or inhibit the remodelling step, which would leave the mRNA attached to its transcription site as a target for exosome-mediated degradation.

Although it is difficult to distinguish transcription site-associated degradation from nucleoplasmic degradation, current data suggest that polyadenylated, as opposed to unadenylated, mRNAs are preferentially stabilized in the absence of Rrp6p (Hilleren et al., 2001; Libri et al., 2002; Torchet et al., 2002). How is degradation normally triggered? A clue might come from an unlikely source: prokaryotes and prokaryote-like organelles. In contrast to eukaryotic cells where poly(A) tails are stabilizing, polyadenylation in bacteria, mitochondria, and chloroplasts accelerates RNA degradation (Dreyfus and Regnier, 2002). In *E. coli*, this polyadenylation-dependent RNA decay is carried out by a multiprotein complex called the degradosome. It contains polynucleotide phophorylase (PNPase), suggested to be the prokaryotic equivalent of the eukaryotic exosome (Dreyfus and Regnier, 2002). The bacterial poly(A) polymerase, PAP, is functional in a complex with the degradosome. In addition to destabilizing mRNA, the PAP/PNPase complex also exercises quality control and degrades otherwise stable *E. coli* RNAs that are misfolded (Li et al., 2002). Exonucleolytic decay is triggered by PAP-mediated poly(A)-addition and then catalyzed by PNPase and RNaseII, a putative equivalent of the eukaryotic deadenylase. It is therefore tempting to suggest a model for eukaryotic transcription-site mRNP surveillance, inspired by the results from *E. coli* (Figure 3). The presence of both Pap1p and the exosome creates a molecular environment at eukaryotic transcription sites that resembles that of the *E. coli* protoplasm. Perhaps competition between Pap1p-dependent polyadenylation and 3′–5′ degradation by the exosome (or associated deadenylases) creates a situation where only healthy mRNPs have an adequate complement of protective proteins bound to the poly(A) tail to fend off exonucleolytic attack (Figure 3). In support of such a model is the yeast Rrp6p-Pap1p connection (mentioned above) as well as the observation that deadenylase activities (Ccr4p and Pop2p) are associated with the RNAPII transcription complex in yeast (Tucker et al., 2002).

As with most models, several questions come quickly to mind: (1) What other steps are involved in the degradation process, and how are they orchestrated? Decapping and 5′-3′ exo-nucleolytic activities are presumably required to complete the mRNA degradation process. Whereas these activities are present in nuclei, little is known about their function and interplay with other bet-

ter-studied nuclear activities. (2) What governs the decision between exosome-mediated retention and exosome-mediated degradation? Although it is not known whether these processes are separable, the creation and analysis of exosome mutants might be informative. A starting point would be to assign roles to individual exosome components. For example, differences in processing defects of mature 5.8S rRNA and box C+D snoRNAs are observed in the absence of Rrp6p activity compared to other exosome components. This difference may extrapolate to mRNA processing (Torchet et al., 2002). (3) Identification of additional mRNP features responsible for the retention and/or degradation leg of transcription-site mRNP surveillance is needed. Deletion of the large subunit of the yeast nuclear cap binding complex (Cbp80p) stabilizes nuclear reporter mRNAs, and it has been suggested that Cbp80p defines a degradation pathway that acts on mRNAs partially retained in nuclei (Das et al., 2000). It therefore might be of interest to analyze the cap for a contribution to exosome recruitment and mRNP surveillance. Needless to say, an in vitro system (a daunting prospect to say the least) will aid in testing the validity of any in vivo progress on these fronts.

mRNA synthesis is a potentially wasteful process. As in other cases of mass production, efficient waste control is required. Not all mRNA processing occurs cotranscriptionally, suggesting that a subset of aberrant mRNPs escape transcription-site associated surveillance. These are probably monitored by additional downstream quality control mechanisms. However, it makes sense for the cell to have a major monitoring system connected to transcription, so that early precautions are taken to prevent aberrant mRNP from contaminating the cell and its resources. This is probably also why erroneous processing in some cases dramatically reduce gene activity. It is tempting to speculate that such feedback systems are general and that other improper mRNP formation steps not only result in rapid mRNA decay but also send signals to turn down transcription. Although there is presently no evidence for such a connection, communication between transcription and nascent RNP formation may prove to be yet another gene expression quality control step.

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