Splicing and mRNP (Ribonucleoprotein Complex) Movement

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ABSTRACT
Eukaryotic cells export several different classes of RNA molecule from the nucleus, where they are transcribed, to the cytoplasm, where the majority participate in different aspects of protein synthesis. It is now clear that these different classes of RNA, including rRNAs, tRNAs, mRNAs and snRNAs, are specifically directed into distinct but in some cases partially overlapping nuclear export pathways. All non-coding RNAs are now known to depend on members of the karyopherin family of Ran-dependent nucleocytoplasmic transport factors for their nuclear export. In contrast, mRNA export is generally mediated by a distinct, Ran-independent nuclear export pathway that is both complex and, as yet, incompletely understood. However, for all classes of RNA molecules, nuclear export is dependent on the assembly of the RNA into the appropriate ribonucleoprotein complex, and nuclear export therefore also appears to function as an important proofreading mechanism.

Keywords---- adaptor, mRNA export, nuclear pore complex, splicing, TREX.

I. INTRODUCTION
During expression of protein-coding genes, pre-mRNAs are transcribed in the nucleus and undergo several processing steps, including capping, splicing, 3′-end processing and polyadenylation. The mature mRNA is then exported through nuclear pore complexes (NPCs) to the cytoplasm for translation. Although distinct and highly complex cellular machines carry out each of these steps in the gene-expression process, growing evidence suggests the existence of gene-expression factories in which individual machines are functionally coupled. This coupling is obviously not obligatory, as virtually all of the coupled steps in gene expression can be uncoupled in various assays. Thus, coupling probably exists to enable the proofreading and streamlining of the entire process of gene expression in vivo. In this review, the discussion will focus on advances made in the past year in elucidating the mechanisms coupling splicing and transcription to mRNA export. In addition, new studies that link the nuclear exosome to the machineries involved in transcription, 3′ end formation and mRNA export will be discussed. Readers are referred to several recent reviews for more extensive descriptions of coupling between the different steps in gene expression [3] [18] [23] [28] [29].

II. TREX IS LOADED DURING SPLICING
In contrast to yeast, studies in mammals indicate that the TREX (Transcription Export) complex is recruited to mRNA during splicing. Early evidence for this possibility came from the observation that both UAP56 and Aly co-localize with splicing factors in nuclear speckle domains [30]. Additional work showed that these proteins are associated with the spliceosome [19] and are recruited to mRNA during a late step of splicing in vitro. Several additional proteins, most of which function in nonsense-mediated decay, are also recruited to mRNA during splicing. These proteins associate with spliced mRNA near exon–exon junctions in a specific complex known as the exon junction complex (EJC) [10][11][18][39]. Now, striking results have been obtained in vivo in mammalian cells indicating that recruitment of UAP56 and Aly (and EJC proteins) to nascent transcripts depends on splicing [8]. Using in situ hybridization and confocal microscopy, Carmo-Fonseca and colleagues [8] showed that Aly and UAP56 co-localize with spliceosome components at sites where nascent wild-type β-globin transcripts are synthesized. By contrast, the mRNA export receptor protein TAP was not detected at these sites. Importantly, when a mutant β-globin gene encoding a pre-mRNA incapable of splicing was used in the same assay, not only were the spliceosome components not detected at the site of transcription, but neither were UAP56 and Aly. Thus,
the implication of the study is that the export machinery is recruited to the site of transcription in a splicing-dependent manner.

Sub2 and Yra1 are recruited to mRNA co-transcriptionally by the THO complex. So, the question is how UAP56 and Aly are recruited. New studies in mammals indicate that, as in yeast, UAP56 and Aly are recruited to mRNA by a stable complex, and this complex is the apparent counterpart of the yeast THO complex. The *Drosophila* and human THO complexes were recently characterized, and both were shown to contain homologs of yeast Tho2 and Hpr1 [31][36]. Three other components of the human THO complex, fSAPs 79, 35 and 24, have counterparts in *Drosophila* (THOC5, 6 and 7, respectively), but these are not present in yeast [31][42]. Conversely, neither the *Drosophila* nor the human THO complexes contain homologs of Mft1 or Thp2. Despite the differences in composition, recent *Drosophila* RNA interference studies of dTho2 and dHpr1 indicate that the metazoan THO complex, like its yeast counterpart, functions in mRNA export [31].

Unexpectedly, recent work indicates that, unlike in yeast, the mammalian THO complex does not appear to be directly linked to the transcription machinery, but instead may be coupled to the splicing machinery. In particular, all of the components of the human THO complex are associated with purified spliceosomes [42][19]. Moreover, hTHO components associate with spliced mRNA, but not with unspliced pre-mRNA.

A model for recruitment of the metazoan TREX complex. Abundant evidence exists that splicing occurs co-transcriptionally in metazoans [44]. Thus, if recruitment of the TREX complex occurs during splicing then it is also a co-transcriptional event. It is noted that, in contrast to the direct co-transcriptional recruitment seen in yeast, the recruitment of the TREX complex in metazoans would be indirect and occur via splicing.

### III. mRNPs MOVEMENT

One major difference between transport of mRNP through the NPC and karyopherin-mediated transport is the size of the cargo. Among the largest mRNPs are the Balbiani ring granules from the dipteran *Chironomus tentans*. These granules contain 30–40-kb mRNAs plus mRNP proteins, are expected to have a mass of >50 MD, and are approximately twice the diameter of the NPC channel. Therefore, it is essential that they become at least partially unfolded in order to pass through the NPC. Electron microscopy and high resolution scanning electron microscopy have revealed BR granule mRNPs caught during transit through the NPC channel [9]. By analyzing asymmetric features of the BR mRNP, it was determined that these mRNPs pass through NPCs with their 5′ end entering the channel first. Key contacts occur between the 5′ end of the mRNP and the basket of the NPC and the mRNP then appears to dock at the entrance to the central channel. As it enters the cytoplasm, ribosomes could be seen binding to the emerging mRNP. We do not know if all mRNAs interact with the NPC in the same way as BR granules, though it is reasonable to hypothesize that mRNPs share this 5′-to-3′ polarity of movement through the channel.

In studies utilizing single molecule fluorescence microscopy, it was demonstrated that karyopherins complexed with the cargo they transport move randomly via diffusion within the channel of the pore, where they presumably make multiple sequential contacts with the FG repeats that line the NPC channel [40]. The rate-limiting step for crossing the nuclear envelope appears to be escape of the complex from the channel. It is thought that export of proteins and small RNAs, which are karyopherin-mediated, will show similar behavior when analyzed at the single-molecule level.

Because of their configuration and much greater size, it is unlikely that mRNPs are able to use diffusion to traverse the channel. Because they are not rigid, it seems mechanistically unlikely that they could be pushed through the NPC from the nuclear side. It is not known precisely how transport of mRNPs takes place, several studies support the hypothesis that mRNPs are pulled through the NPC channel and that this movement is powered by ATP hydrolysis. In support of this model, an ATPase, the DEAD-box protein Dbp5, binds to the cytoplasmic filaments of the NPC, positioning it to interact with the emerging mRNP [15][33].

### IV. ADAPTER COMPLEX

Although TAP is able to interact directly with RNA in a sequence-nonspecific fashion through its non-canonical RNP domain [21], this region of TAP is not essential for mRNA export in vivo [4]. This observation suggested that RNA-binding proteins are needed to bridge the interaction between TAP and mRNA. Using both genetic [35] and biochemical [37] approaches, the essential protein Yra1p was identified as a Mex67p-binding partner. The *YRA1* gene encodes an RNA binding protein, which when deleted causes the nuclear accumulation of poly(A)+ mRNA [35] and [37] implicating Yra1p in mRNA export. The genetic and biochemical data combined suggest that Yra1p is an mRNA export adapter for Mex67p. Proteins homologous to Yra1p have been identified in human, mouse, *C. elegans*, *Xenopus laevis*, and *Drosophila*, and belong to an evolutionarily conserved protein family called RNA export factor binding proteins or REFs [35][38].

Metazoan REF interacts directly with TAP (Stutz et al., 2000). Several lines of evidence suggest that REF, like Yra1p, is an mRNA export adapter. Initial investigations showed that REF associates with vertebrate mRNAs during splicing [43] and could stimulate the export of spliced mRNAs. Subsequently, REF was shown to
stimulate the export of intronless mRNAs in Xenopus oocytes. Interestingly, though, in Drosophila S2 cells, depletion of the sole REF does not result in the nuclear accumulation of poly(A)⁺ mRNA [13]. Likewise, REFs are dispensable for mRNA export in C. elegans [22], suggesting that TAP association with mRNAs may occur through other adapter proteins in addition to REF. This view is supported by the recent identification of additional TAP-binding partners that include several shuttling SR proteins [16].

V. ALTERNATIVE EXPORT OF mRNAs

Recently, it was shown that mRNPs that are too large to translocate through the center channel could bypass the NPC by using a mechanism similar to the Herpes virus nuclear egress [14][24][25][26][34]. This process is termed nuclear envelope budding. It was shown that during synapse development, large mRNP granules exit the nucleus by budding through the nuclear envelope (NE). This budding involves phosphorylation of the nuclear lamin by an atypical protein kinase C [34]. The phosphorylated lamin permits the invagination of the inner nuclear membrane (INM) into the NE lumen. Then a vesicular fusion with the outer nuclear membrane (ONM) permits the release into the cytoplasm. This process permits the export of large mRNPs without remodeling of the NPC [34].

Another interesting RNP export modality was discovered while investigating mRNA export in Influenza A viruses (IAV) [32]. Electron microscopy studies show that IAV enlarges the nuclear pores in infected cells by around 20 nm (a final size of around 50 nm) [12][27]. The increase in pore diameter facilitates the translocation of large protein complexes. This widening is due to a virus-induced cellular caspase activity. Newly synthesized viral RNPs normally use the CRM1 transporter with the viral structural nucleoprotein NP as an adaptor. However the enlargement of the nuclear pore seems to dramatically change the diffusion limits of NPCs at late infection stages, since proteins of ~125 kDa are able to accumulate in the cytoplasm via passive diffusion [27]. This allows the passive diffusion of viral RNPs which can complement CRM1-dependent RNP export mechanisms to increase the production of infectious virus progeny at late stages of the replication cycle [12]. Future studies will determine whether this mechanism is also relevant to host cells.

VI. mRNP SURVEILLANCE AND ASSEMBLY

Recent data from the Jensen, Libri, and Rosbash labs [20] as well as work by other groups [41] have linked TREX to the nuclear exosome, a large complex of 3'-to-5' exonucleases involved in RNA processing and degradation of improperly assembled mRNP particles or mRNP particles whose 3' ends have not been properly processed. These two studies show that mutations in THO, Sub2p or Yra1p result in low mRNA levels and the sequestration of newly made transcripts in nuclear foci. Importantly, the deletion of Rrp6p, a nuclear exosome component, releases transcripts from nuclear dots and rescues the truncation phenotype of TREX mutants, indicating that transcripts are made in these strains but retained and degraded by the exosome. These observations suggest that THO, Sub2p and Yra1p function primarily in co-transcriptional mRNP assembly, ensuring packaging of mRNA into stable and exportable mRNP particles. In situ localizations by the Jensen lab recently showed that transcripts retained in foci are in close proximity to the encoding locus. Consequently, the nuclear exosome was proposed to monitor early mRNP assembly, retaining and eliminating improperly 3' processed or malformed mRNP particles at or close to the site of transcription. Strong evidence for co-transcriptional monitoring of mRNP assembly came from the Lis lab, who showed that in Drosophila the exosome is recruited to active genes via interactions with elongation factors [2].

The observation that transcripts made in TREX mutants are degraded by the exosome is subject to controversy. Long-standing work from the Aguilera lab claims that mutations in THO affect transcription elongation, especially of long and GC-rich mRNAs such as LacZ, and that these elongation problems in turn cause DNA hyper-recombination and genome instability [5][6][7]. This lab now provides a molecular mechanism for the proposed elongation defect [17]. They show that nascent transcripts produced in a THO mutant tend to form DNA:RNA hybrids with the transcribed region behind the advancing polymerase. Importantly, ribozyme-mediated self-cleavage of the nascent mRNA and over-expression of RNase H1 eliminate DNA:RNA hybrids, and concomitantly rescue the elongation and hyper-recombination phenotypes. The authors propose that the DNA:RNA hybrids directly affect transcription elongation by obstructing the next elongating polymerase. This roadblock may in turn favor DNA hyper-recombination, linking efficient mRNP packaging to genome stability [1]. An important role of THO/TREX may therefore be to prevent DNA:RNA hybrid formation, probably by promoting efficient co-transcriptional mRNP packaging.

VII. CONCLUSIONS

mRNA transcription and export constitute a chain of molecular events offering many points of control. Although there are many combinations of promoter–transcription-factor associations, the downstream cellular responses cannot be explained by promoter firing alone. To understand the subtleties of these transcriptional
pathways, single cell approaches are necessary. Recent developments in our ability to probe single cells in real time have yielded new information on the dynamics of gene expression. These studies will ultimately take us to the complex task of unraveling the dynamics of transcription within the live organism.

Although several components involved in the export of mRNA, tRNA, and rRNA have been identified, the mechanism of RNA export (in particular for mRNA and rRNA) is not understood and many questions remain open. How is an RNA recognized as fully processed and therefore made export-competent? How do nuclear export factors interact with transport-competent hnRNPs and what are the nuclear export signals (on the RNA, the proteins, or both)? Which components are involved directly in RNA export and which ones play a role in RNA folding and RNP assembly? How does transport occur within the NPC environment and are there delivery routes from the pores to the sites of translation? Thus, it is evident that many questions still remain open in the field of nuclear RNA export through the nuclear pores.

Recent studies strengthen the view that mRNA export is coupled to other steps in gene expression including splicing, transcription, and 3′-end formation. In addition, new links between mRNA export, transcription and the nuclear exosome were revealed. Future directions will include a detailed characterization of the spliced mRNP and EJC that couples splicing to mRNA export. In addition, the role of the TREX complex in export and transcription, and the relationship between these processes and the nuclear exosome remain to be determined. Finally, the molecular basis for coupling 3′-end formation to mRNA export must be identified.

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