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TREX, SR proteins and export of mRNA

Robin Reed and Hong Cheng

The machineries involved in gene expression are highly conserved from yeast to metazoans. However, a fundamental difference between these organisms is that most yeast genes lack introns whereas the converse is true in higher organisms. Recent studies of the TREX complex, which functions in mRNA export, unexpectedly revealed that this complex is recruited by the transcription machinery in yeast whereas the TREX complex appears to be recruited by the splicing machinery in mammals. Studies during the past year also revealed a possible conserved role for SR protein dephosphorylation in regulating the interaction between SR proteins and the mRNA export receptor TAP (Mex67 in yeast). There is also an interesting possibility that an SR protein–TREX complex interaction is a conserved part of the mRNA export machinery.

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Current Opinion in Cell Biology 2005, **17**:269–273

This review comes from a themed issue on
Nucleus and gene expression
Edited by Christine Guthrie and Joan Steitz

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2005.04.011

Introduction

The TREX (transcription/export) complex is a key player in the transport of mRNA from the nucleus to the cytoplasm. This complex is conserved from yeast to human, and a *Drosophila* counterpart was recently characterized. The two main constituents of the TREX complex are the stable multi-subunit THO complex and the mRNA export proteins UAP56 (Sub2 in yeast) and Aly (Yra1 in yeast). As discussed below, the THO complex plays a central role in recruiting these export proteins to the mRNA in both yeast and human. Despite the striking conservation in the structure and function of the TREX complex, studies in yeast provide strong evidence linking the TREX complex to transcription elongation and to co-transcriptional recruitment of the mRNA export machinery. By contrast, studies in mammals provide compelling evidence linking the TREX complex to the splicing machinery and suggesting that that the TREX complex is recruited to mRNA during a late step in splicing.

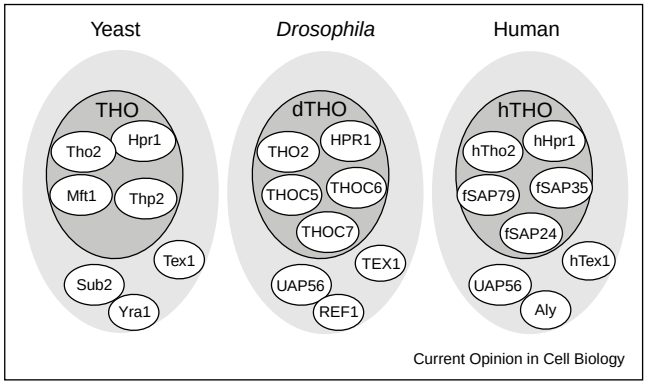
Our review focuses on the evidence for the different TREX complex recruitment mechanisms as well as on the puzzles that remain regarding the export of naturally intronless mRNAs in mammals and spliced mRNAs in yeast. We also discuss exciting new studies of the SR (serine/arginine-rich protein) family of splicing factors in mammals and related proteins in yeast that reveal a role for dephosphorylation of these proteins in mRNA export and the identification of a specific nuclear phosphatase in yeast. For a comprehensive discussion of the coupling between transcription, splicing and mRNA export, readers are referred to excellent new reviews of these topics [1–4].

Co-transcriptional recruitment of the yeast TREX complex

A central component of the TREX complex is the THO complex, which in yeast consists of the proteins Tho2, Hpr1, Mft1 and Thp2 [5] (Figure 1). Studies over the past ten years have indicated that the yeast THO complex functions in transcription elongation [5,6]. More recently, the function of this complex has come into sharper focus. In particular, the THO complex was shown to associate with actively transcribed genes along their entire length, suggesting that it plays a role in transcription elongation [7,8••]. In addition, THO complex mutants form RNA:DNA hybrids between the nascent transcript and the DNA template, and these hybrids inhibit transcription elongation [9]. The THO complex components are thought to function in packaging of nascent mRNA and this packaging may prevent hybrid formation [10]. Studies this past year by Jensen, Libri and co-workers indicate that the rate of transcription can influence packaging of mRNA by components of the THO/TREX complex, which in turn determines whether the mRNP is a target of the nuclear exosome [11•].

The yeast THO complex is also thought to function in the co-transcriptional loading of the mRNA export proteins Sub2 and Yra1 onto nascent transcripts. In a previous study, Stutz and colleagues showed that Hpr1 interacts directly with Sub2 and is required for recruitment of Sub2 and Yra1 to the mRNA [12]. Using chromatin immunoprecipitation in combination with RNase (ChIP/RNase) to analyze a large number of genes, Rosbash and colleagues [8••] have now provided evidence that the THO complex primarily associates with the DNA template, whereas Sub2 is loaded onto the nascent transcript. Yra1 appears to associate with both the DNA and the nascent

Figure 1



The conserved TREX complex. The yeast, *Drosophila* and human TREX complexes contain the THO complex and export proteins, as well as a protein of unknown function known as Tex1. See text for details.

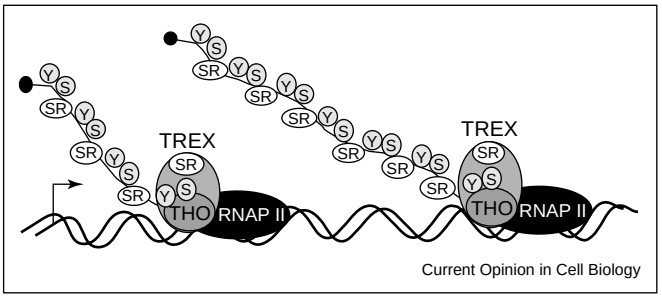
RNA [8••]. As shown in Figure 2, the available data have led to the model that the TREX complex is recruited to active genes and that the THO complex travels with RNAP II and transfers Yra1 and Sub2 to the nascent transcript. The current view is that the entire intronless yeast transcript is coated by Sub2 and Yra1 [8••].

Recruitment of the mammalian TREX complex during splicing

In contrast to yeast, studies in mammals indicate that the TREX 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 (for review, see [13]). Additional work showed that these proteins are associated with the spliceosome (for review, see [14]) and are recruited to mRNA during a late step of splicing *in vitro* (S Masuda, R Das, H Cheng and R Reed, unpublished). 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) (for reviews, see also [1–4]). 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 [15••]. Using *in situ* hybridization and confocal microscopy, Carmo-Fonscea and colleagues [15••] 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.

As described in our discussion of the yeast TREX complex, 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 (S Masuda, R Das, H Cheng and R Reed, unpublished) (Figure 1). The *Drosophila* and human THO complexes were recently characterized (Figure 1), and both were shown to contain homologs of yeast Tho2 and Hpr1 [7,16••] (S Masuda, R Das, H Cheng and R Reed, unpublished). 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 [16••,17] (S Masuda, R Das, H Cheng and R Reed, unpublished). Conversely, neither the *Drosophila* nor the human THO complexes contain homologs of Mft1 or Thp2 (Figure 1). Despite the differences in composition, recent *Drosophila* RNA interfer-

Figure 2



Co-transcriptional recruitment of the TREX complex in yeast. mRNA export proteins Yra1 (Y), Sub2 (S), and SR-like proteins Gbp2 and Hrb1 (SR) associate with the THO complex to form the TREX complex. This complex is recruited to active genes and functions to load Yra1, Sub2 and the SR-like proteins onto the nascent transcript. NP/3, another SR-like protein, is co-transcriptionally loaded onto the mRNA independently of the TREX complex.

ence studies of dTho2 and dHpr1 indicate that the metazoan THO complex, like its yeast counterpart, functions in mRNA export [16••].

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 [17] (for review, see [14]). Moreover, hTHO components associate with spliced mRNA, but not with unspliced pre-mRNA (S Masuda, R Das, H Cheng and R Reed, unpublished).

A model for recruitment of the metazoan TREX complex is shown in Figure 3. Abundant evidence exists that splicing occurs co-transcriptionally in metazoans (for review, see [18]). Thus, if recruitment of the TREX complex occurs during splicing then it is also a co-transcriptional event. But we note that, in contrast to the direct co-transcriptional recruitment seen in yeast (Figure 2), the recruitment of the TREX complex in metazoans would be indirect and occur via splicing (Figure 3).

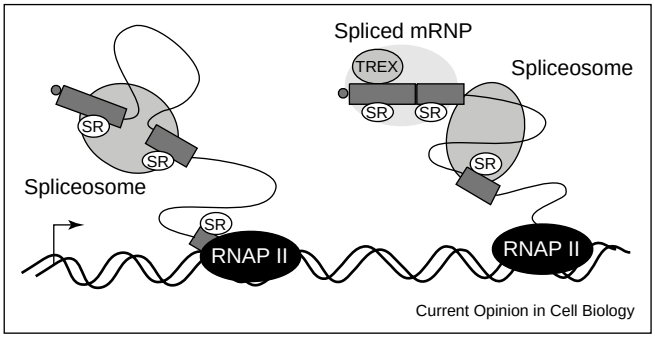
A conserved role for SR proteins in mRNA export

Although this review has focused on the conserved TREX export machinery, proposals have been made that there are multiple physiologically relevant adaptors for mRNA export distinct from TREX [19]. These adaptors have been proposed based in part on the observation that Aly, UAP56 and the THO complex can all be supplanted by other pathways in *Drosophila* RNAi studies [16••,20]. Although other adaptors have been proposed, the only general candidates so far reported are members of the SR family of splicing factors [21]. In metazoans, these proteins bind to exon sequences in pre-mRNA and recruit the spliceosome to the flanking 5′ and 3′ splicing sites (for review, see [22]). After splicing, SR proteins remain bound to the spliced mRNA (for review, see [23]). SR proteins also shuttle between the nucleus and cytoplasm, as would be expected of an mRNA export protein [24]. More recent studies showed that SR proteins mediate mRNA export [21,25]. Moreover, the SR proteins involved (9G8 and SF2) function by direct interaction with TAP [25]. SR proteins are known to be serine-phosphorylated, and the phosphorylation state is regulated during splicing (for review, see [23]). Now Steitz and co-workers have obtained interesting new data showing that phosphorylation of SR proteins is also involved in regulating the interaction between these proteins and TAP [26••]. Specifically, the dephosphorylated form of SR proteins associates with mRNA and with TAP and thus this dephosphorylation is thought to be a mechanism for the selective export of spliced mRNA versus unspliced pre-mRNA [26••].

In yeast, Npl3 is an SR-like shuttling protein that has no known role in splicing but functions in mRNA export [27]. This protein is co-transcriptionally recruited to active genes and interacts with RNAP II [27]. Now Guthrie and colleagues have obtained exciting new data suggesting that dephosphorylation of SR proteins to stimulate their interaction with mRNA and with the mRNA export receptor may be conserved from yeast to human [28••]. Specifically, these workers showed that a nuclear phosphatase, Glc7p, is required for mRNA export. Their data indicate that Glc7p functions to dephosphorylate Npl3, which results in mRNA becoming dissociated from the 3′-end-processing machinery and instead associating with the mRNA export receptor Mex67 [28••]. It will be interesting to determine whether a related phosphatase is involved in SR protein dephosphorylation in higher eukaryotes and whether release of mRNA from the 3′ processing machinery is also involved in mRNA export.

In contrast to Npl3, which does not associate with the TREX complex, Gbp2 and Hrb1 are SR-like yeast pro-

Figure 3



Recruitment of the human TREX complex during splicing. Spliceosome assembly and splicing occur as the transcript is synthesized by RNAP II. SR proteins (SR), which recruit the spliceosome, remain bound to the spliced mRNA, and the TREX complex is recruited to the spliced mRNA. For simplicity, other components of the spliced mRNP, such as EJC proteins, are not shown.

teins that do associate with the TREX complex and require THO complex components for export [29•,30•]. ChIP and RNA immunoprecipitation studies show that these proteins are associated with actively transcribed genes throughout their lengths and are bound to nascent transcripts (Figure 2). Thus, SR-like proteins and TREX may function together in mRNA export, though a direct role for Gbp2 and Hrb1 in mRNA export has not yet been detected. Considering that SR proteins and the TREX complex are both bound to spliced mRNA in mammals (Figure 3), it would be interesting to determine whether there is an association between SR proteins and the mammalian TREX complex as in yeast.

A puzzle: recruiting the mRNA export machinery to intron-containing transcripts in yeast

Although few yeast genes contain introns, those genes that do are highly expressed. Thus, export of spliced mRNA is a major process in yeast. How the export machinery is loaded onto intron-containing genes and their transcripts was also investigated by Rosbash and colleagues in their recent work [8••]. Using ChIP/RNase assays, they found different results depending on the structure of the gene. In particular, when the intron was located close to the 3′ end of the gene, Sub2 and Yra1 appeared to bind in the same manner as observed with intronless genes, associating all along the transcript upstream of the intron. However, with introns located close to the 5′ end or in the middle of the gene, the data suggest that Yra1 and Sub2 are not associated with the region of the transcript where the spliceosome binds. Thus, these data led to the view that the export machinery is excluded from the pre-mRNA by the spliceosome. The authors propose that this may be one mechanism for preventing export of unspliced pre-mRNAs. The exclusion of the mRNA export machinery from the unspliced pre-mRNA also occurs in mammals, as noted above. An unanswered question is whether the yeast spliceosome subsequently plays a role in recruiting the export machinery to the spliced mRNA as occurs in mammals or whether the export machinery is recruited to the spliced mRNA by some other mechanism after the spliceosome is released.

Another puzzle: recruiting the mRNA export machinery to intronless mRNAs in metazoans

The converse problem exists in metazoans: how is the export machinery recruited to mRNAs derived from naturally intronless genes? The SR proteins 9G8 and SRP20 are not only thought to function in the export of spliced mRNA but also to function in the export of intronless mRNA [21,25]. In previous work, Steitz and colleagues showed that these two SR proteins bind to a specific element within the naturally intronless histone *H2a* mRNA and mediate its export [21]. Whether SR proteins function in export of other intronless mRNAs and how they are recruited to those mRNAs is not known. Neither is it clear whether the TREX complex has a general role in the export of naturally intronless mRNAs in metazoans as it does in yeast. In support of this possibility, Izaurralde and co-workers showed that TREX complex components are involved in export of some mRNAs derived from naturally intronless mRNAs in *Drosophila* [16••,20]. Thus, a splicing-independent mechanism must exist not only for SR protein recruitment but also for TREX complex recruitment to mRNA.

Conclusions

The TREX complex is conserved from yeast to human and functions in mRNA export. Despite this conservation, the complex appears to be recruited to mRNA by the transcription machinery in yeast and the splicing machinery in human. It makes more sense to load the export machinery onto spliced mRNA than onto unspliced pre-mRNA in the case of higher eukaryotes, which have numerous introns. And, conversely, it makes sense in yeast to make use of the transcription machinery for loading the export machinery, as most transcripts are not spliced. The obvious question raised is how to deal with the intron-containing genes in yeast and the intron-lacking genes in higher organisms. Studies over the past year have revealed a conserved role for dephosphorylation of SR proteins in mRNA export, but how they, the TREX complex, or some as yet undetected adaptors interact and function in these processes remains to be determined.

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