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mRNA export: an assembly line from genes to nuclear pores

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mRNAs are transported from the nucleus to the cytoplasm by a machinery conserved from yeast to humans. Previous studies showed that mRNA export factors are loaded on nascent mRNAs during elongation, coupling transcription to export. More recently identified mRNA export factors connect transcription initiation to the export machinery associated with nuclear pores, and potentially tether active genes to the nuclear periphery. Furthermore, a newly identified link between the nuclear exosome and the transcription, 3′-end formation and export machineries suggests that early messenger ribonucleoprotein complex (mRNP) assembly is co-transcriptionally monitored. Moreover, inefficient mRNP assembly impairs transcription elongation, indicating tight interdependence of these processes. Finally, nuclear retention of unspliced mRNAs by the perinuclear Mlp proteins reveals a novel mechanism of mRNP surveillance prior to export.

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Abbreviations

BA	boundary activity
EJC	exon junction complex
mRNP	messenger ribonucleoprotein complex
NPC	nuclear pore complex
Pol II	RNA polymerase II
RNAi	RNA interference
TREX	transcription and export complex

Introduction

Considerable evidence indicates that the different steps involved in the biogenesis and export of mRNAs are tightly linked to each other. RNA polymerase II (Pol II) plays a central role in all these events, as it mediates the recruitment to nascent mRNAs of factors involved in 5′ capping, splicing, 3′ end formation and mRNA export [1,2]. The deletion of the yeast RNA polymerase II C-terminal domain (CTD) results in cleavage and polyadenylation defects, and mutations in the 3′ end processing machinery interfere with transcription termination and export [3–5]. Conversely, defects in RNA processing can feed back on the pathway and negatively influence transcription [6,7]. All these molecular couplings are likely to be part of surveillance mechanisms ensuring that only fully mature and functional messenger ribonucleoprotein (mRNP) particles reach the cytoplasm [8,9].

In this review, we first focus on newly identified and evolutionarily conserved mRNA export factors, which extend the links between the transcription and mRNA export machineries, and suggest that multiple, possibly overlapping, pathways direct mRNPs to the cytoplasm. We then address the role of post-transcriptional modifications in the regulation of mRNA export. Finally we discuss the early steps of mRNP assembly and the surveillance mechanisms developed by the cell to prevent accumulation and export of malformed and non-functional mRNP complexes.

mRNA export receptors and adaptors

Major constituents of the mRNA export pathway have been identified and appear to be conserved from yeast to humans. The mRNA export adaptor Yra1p/REF (Aly/REF in mammals) and its partner, the ATPase/RNA helicase Sub2p (UAP56), associate with mRNA during transcription. At a later stage, Yra1p/REF recruits the essential heterodimeric export receptor Mex67p–Mtr2p (TAP/NXF1–p15/NXT1). Mex67p–Mtr2p releases Sub2p and promotes mRNP translocation by mediating interactions with nucleoporins lining the pore [9,10].

Recent RNAi-based knock-down experiments in *Drosophila* cells by the Izaurralde lab revealed that whereas NXF1 and UAP56 are required for the export of most transcripts, REF is not essential for bulk poly(A)+ RNA export [11•,12,13•]. REF proteins are also dispensable in *Caenorhabditis elegans* [14•]. These data suggest that additional adaptors mediate the interaction between TAP/NXF1 and cellular mRNAs in metazoans, and that the essential role of UAP56 in mRNA export may not be restricted to the recruitment of REF. Steitz and co-workers presented evidence that the serine/arginine-rich splicing factors Srp20 and 9G8, previously shown to promote export of intronless histone mRNAs in Xenopus oocytes, bind TAP in the same domain as REF, suggesting that these proteins may function as alternative adaptors [15]. A global approach in yeast, published by the Silver lab, showed that only a fraction of transcripts associate with Yra1p/REF, suggesting the existence of additional adaptors in this lower eukaryote [16•]. Consistently, a recent study by the Guthrie lab shows that the SR-like protein Npl3p directly interacts with the export receptor Mex67p and participates in its recruitment to the mRNP [17••].

In higher eukaryotes, pre-mRNA splicing results in the deposition of the exon junction complex (EJC) on spliced mRNA [18]. The presence of UAP56 and Aly/REF within the EJC and the observation that pre-mRNA splicing enhances the export of certain transcripts in Xenopus oocytes led to the proposition that the EJC couples splicing to export by facilitating the recruitment of TAP/NXF1 to spliced transcripts [10,19]. However, although the presence of an intron may stimulate export of small transcripts, splicing is not a general requirement for nuclear exit. Accordingly, Sub2p/UAP56 and yeast Yra1p are essential for the export of transcripts derived from intron-containing and intron-less genes, indicating that these essential export factors can bind mRNAs via a mechanism independent of splicing [20]. Recent studies by the Moore and Cullen labs further demonstrated that pre-mRNA splicing does not promote mRNA export in mammalian cells, but rather that EJC components enhance 3′-end processing/polyadenylation and mRNA polysome association, resulting in increased translation of spliced versus intronless transcripts [21•,22•].

mRNA transcription and export are functionally coupled

Early experiments by the Silver lab have shown that Yra1p/REF is recruited to mRNA during transcription [23]. More recent genetic and biochemical interactions published by the Hurt and Jensen labs and our lab have connected Sub2p and Yra1p to THO, a tetrameric complex composed of Hpr1p, Tho2p, Mft1p and Thp2p. THO has been implicated in transcription elongation and genome stability and more recently in mRNA export [24,25•, 26••,27•]. It is unclear how conserved this complex is, as there are no clear homologues of Mft1p or Thp2p in metazoa or even in *S. pombe*. THO, Sub2p and Yra1p co-purify in a complex called TREX, which is proposed to couple transcription and export. TREX components are recruited to an active gene at a similar time during transcription elongation [26••]. Hpr1p directly interacts with Sub2p and facilitates the binding of Sub2p and Yra1p to nascent transcripts [27•].

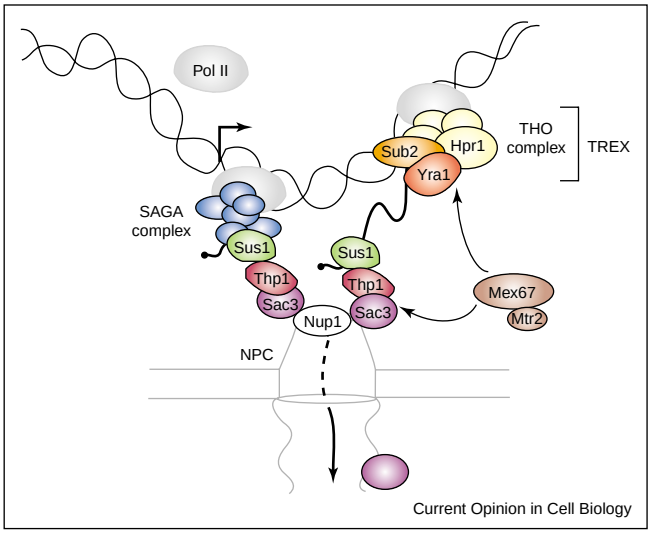
Recent studies by Hurt and co-workers identified additional conserved mRNA export factors linked to the transcription machinery. A screen for mutations synthetically lethal with Yra1p identified Sac3p, a protein interacting with Mex67p and nucleoporins [28•]. Sac3p also tightly binds Thp1p, which was previously implicated in transcription elongation and genome stability [29]. Deletion of either Thp1p or Sac3p results in poly(A)+ RNA export defects. Importantly, Sac3p tethers Thp1p to the nuclear periphery via interaction with FG-nucleoporins on the nuclear side of the nuclear pore complex (NPC) [28•]. In contrast, genetic analyses and immuno-electron microscopy studies by the Silver lab indicated that Sac3p associates with the cytoplasmic face of the NPC and showed that Mex67p accumulates at the nuclear rim when *SAC3* is mutated [30]. This study suggested a role for Sac3p in releasing Mex67p from the mRNP at a terminal step of mRNA export. Considering both the Hurt and Silver studies, it is possible that Thp1p–Sac3p associates with the mRNP at an early stage, and participates in the docking and subsequent translocation of Mex67p-containing mRNPs through the NPC. The Thp1p–Sac3p complex may functionally overlap with Yra1p in linking intranuclear mRNA biogenesis and export.

More recently the Hurt lab described a new protein, Sus1p, which tightens the connection between Sac3–Thp1 and the transcription machinery [31••]. Sus1p associates with the Thp1–Sac3 complex as well as with SAGA, a large intranuclear histone acetylase complex involved in transcription initiation of a subset of Pol II genes [32]. Sus1p is a nuclear protein that concentrates at pores, and its peripheral localization depends on Sac3p. Consistent with its binding to SAGA, Sus1p is recruited to SAGA-dependent genes during transcription initiation. The deletion of Sus1p causes both transcriptional and mRNA export defects consistent with a role in both processes. Moreover, a fraction of Thp1p co-purifies with SAGA, suggesting that Sus1p may physically connect SAGA to the Thp1p–Sac3p complex (Figure 1). The identification of such a supercomplex led the authors to propose that Sus1p couples transcription and export. However, this study failed to provide evidence for a functional coupling between these complexes. Indeed, macroarray analyses identified a modest overlap between the set of genes regulated by the SAGA complex and by Sus1p. In addition, there was no evidence indicating that genes regulated by Sus1p fail to be exported in the absence of Thp1p, Sac3p or a functional Mex67p protein. The modest correlation observed so far between the biochemical and functional data suggests that the proposed role of Sus1p in functionally coupling mRNA transcription and export may concern a limited number of genes. One possibility is that Sus1p recruits the Thp1p-Sac3p complex to a subset of SAGA-dependent promoters, facilitating its interaction with nascent transcripts. Alternatively, Sus1 may tether SAGA-dependent genes to the nuclear periphery via binding to the NPC-bound Thp1–Sac3p complex, optimizing the access of newly made transcripts to the NPC channel. In summary, the current data suggest that TREX (transcription and export complex) and the Sus1p–Thp1p–Sac3p complex represent parallel pathways linking transcription to the export machinery. Whether Thp1–Sac3p and TREX components bind distinct mRNAs or are sequentially recruited to the same transcript remains to be determined (Figure 1).

Regulation of mRNA export

An important question is how mRNA export is regulated and how assembly and disassembly of export complexes is

Figure 1



Model for transcription coupled export by the Sus1–Thp1–Sac3 and THO–Sub2–Yra1 (TREX) complexes. Sus1p connects the SAGA complex, which is involved in transcription initiation, to Thp1p–Sac3p, a complex associated with the NPC via Nup1p. Sus1p may recruit Thp1p–Sac3p to SAGA-dependent transcripts (not shown); alternatively it may tether SAGA-dependent genes to the nuclear periphery at an early stage of transcription. Sac3p may facilitate the docking of mRNP complexes to the NPC via interaction with both Mex67p and nucleoporins [28•,31••]. The mRNA export factors Sub2p and Yra1p are loaded on nascent mRNAs during transcription elongation in a process facilitated by the THO complex, associated with RNA pol II [26••,27•]. Sub2p and Yra1p recruit the export receptor Mex67p, which targets mRNPs through the NPC. Whether Mex67p is recruited by Yra1p and then binds Sac3p, or whether Mex67p is independently recruited by the two coupling factors, is unknown. The shuttling SR-like protein Np13p acts as an adaptor for Mex67p [17••] (not shown).

controlled. Interestingly, Tom1p, an ubiquitin E3 ligase associated with the SAGA complex [33], is required for mRNA export. Indeed, mutations in Tom1p block the export of transcripts containing the shuttling mRNA binding protein Nab2p [34,35]. Genetic interactions further indicate a connection between Nab2p and the Sus1p–Thp1p–Sac3p complex, suggesting that post-translational modification by ubiquitin may regulate this pathway [31••,36]. Recent evidence by the Hurt and Dargemont labs shows that another ubiquitin E3 ligase, called Rsp5p, is also essential for mRNA export [37,38]. The identification of specific substrates should indicate whether Rsp5p and Tom1p control distinct pathways and reveal how ubiquitin regulates mRNP biogenesis and export.

A recent paper by the Guthrie lab demonstrates that post-transcriptional modification by phosphorylation also contributes to mRNA export regulation [17••]. As mentioned above, this study identified Npl3p, an SR-like protein essential for mRNA export, as a new adaptor for the export receptor Mex67p. Npl3p is recruited to nascent mRNPs in its phosphorylated form but interacts with Mex67p only in its unphosphorylated form. Importantly, the authors show that Glc7p, a phosphatase essential for mRNA export, coordinates dephosphorylation of Npl3p with the release of the mRNP from the 3′-end processing machinery and the recruitment of Mex67p to the mRNP. Such a mechanism may ensure that only correctly 3′-end processed mRNPs become associated with the export receptor. After translocation, the cytoplasmic kinase Sky1p phosphorylates Npl3p, promoting the dissociation of Mex67p from the mRNP [39]. These observations show that a cycle of cytoplasmic phosphorylation and nuclear dephosphorylation of Npl3p, and perhaps other shuttling SR proteins, regulates Mex67p-dependent mRNA export [40].

Links between mRNA 3′ end formation and export

Ample evidence indicates that the export machinery is loaded on the mRNA co-transcriptionally. However, 3′ end cleavage and polyadenylation appear to be the most crucial steps for the acquisition of export competency. Both cis- and trans-acting 3′ end processing mutants block mRNA export [41,42,43•]. Moreover, Rosbash's lab showed that transcripts generated by T7 RNA polymerase are exported provided they are correctly processed at their 3′ end. This indicated that 3′ end processing is both necessary and sufficient for export and that export factors can be recruited to mRNAs independently of RNA polymerase II [44•]. The poly(A) binding protein Pab1p and the shuttling-mRNA-binding protein Nab2p contribute to poly(A) tail length control and export, possibly linking the 3′ end processing and export machineries, but the molecular interactions underlying this functional coupling remain poorly defined [35,43•,45•,46]. In contrast, connections between 3′-end formation and transcription are better understood and are discussed by Proudfoot in a review in this issue.

Early mRNP assembly and surveillance

Recent data from the Jensen, Libri, and Rosbash labs [25•] as well as work by our group [27•] 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 3′ end 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 [47•]. 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 [48••].

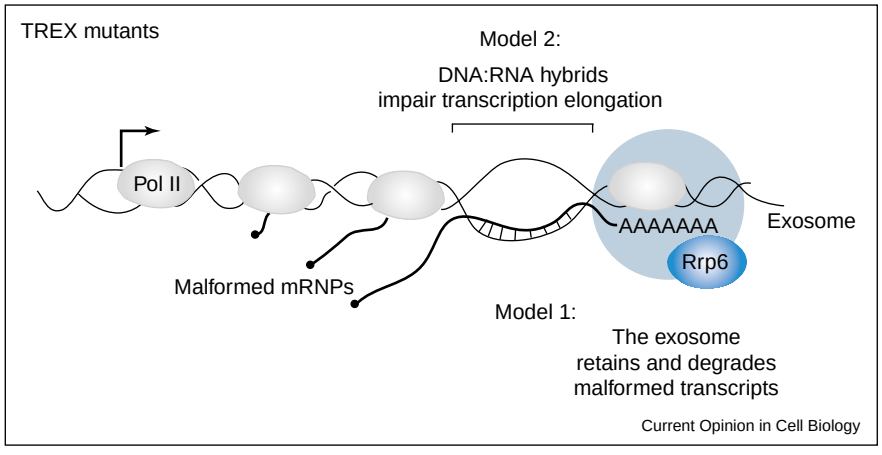
The observation that transcripts made in TREX mutants are degraded by the exosome is subject to controversy. Long-standing work from the Aguilera lab claims that mutations in THO affect transcription elongation, especially of long and GC-rich mRNAs such as LacZ, and that these elongation problems in turn cause DNA hyper-recombination and genome instability [24,49,50]. This lab now provides a molecular mechanism for the proposed elongation defect [51••]. 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 road-block may in turn favor DNA hyper-recombination, linking efficient mRNP packaging to genome stability [52]. An important role of THO/TREX may therefore be to prevent DNA:RNA hybrid formation, probably by promoting efficient co-transcriptional mRNP packaging.

In summary, the 'transcriptional' and 'exosomal' models agree on a primary role for THO in mRNP assembly, but provide different explanations as to the origin of 3′-end-truncated transcripts in THO mutants. The two views are not mutually exclusive and more detailed analyses may reveal that both transcription elongation and mRNA stability are affected by inefficient mRNP assembly (Figure 2). In an extreme scenario, the exosome may itself contribute to polymerase stalling by recognizing DNA:RNA hybrids. More detailed analyses are required to dissect the exact relationship between the transcription machinery, the nascent mRNP and the exosome carrying out surveillance.

Noteworthily, Aguilera and co-workers have shown that mutations in most factors involved in mRNP biogenesis and export, including Sub2p, Yra1p, Thp1p-Sac3p, Nab2p, Mex67p and Mtr2p, confer a transcription elongation defect and transcription-dependent hyper-recombination comparable to that described for THO mutant strains [36,53•]. The similarity of these phenotypes suggests that any problem along the mRNP export assembly line negatively impacts on mRNA synthesis and/or stability, as well as on genome stability. These observations once more underline the strong interdependence of mRNP biogenesis steps from genes to nuclear pores.

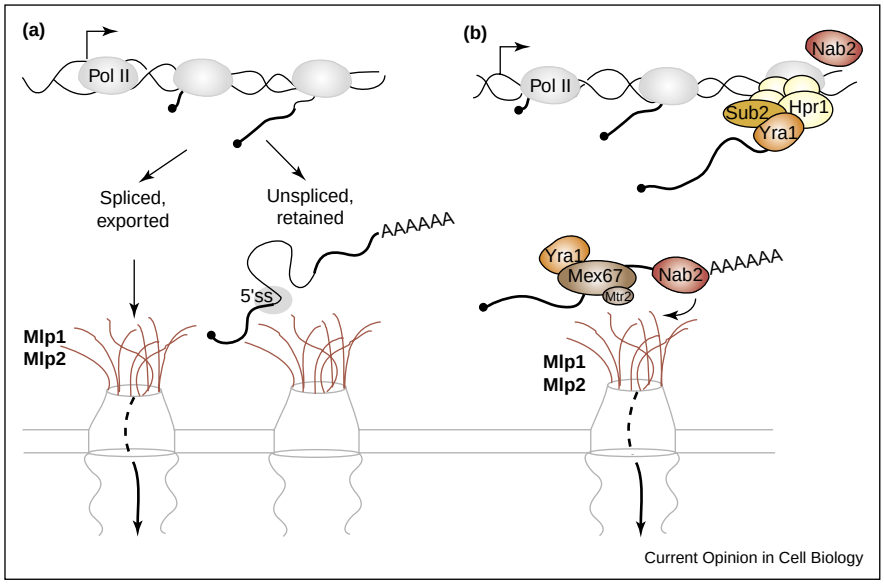
A paper by the Cole lab further illustrates the tight links between mRNP formation and transcription. These

Figure 2



Non-exclusive models to explain low levels of 3′-truncated transcripts in TREX mutants. The exosomal model (model 1) proposes that co-transcriptional surveillance by the nuclear exosome results in the retention and 3′–5′ degradation of malformed mRNPs at or close to the site of transcription. In this view, transcripts are fully synthesized but unstable and degraded [25•,27•]. The transcriptional model (model 2) claims that malformed mRNP complexes form stretches of DNA:RNA hybrids with the coding DNA strand. The DNA:RNA hybrids impair transcription elongation by creating an obstacle for the next elongating RNA polymerase [51••]. Both views propose that an important role of THO/TREX is to promote efficient mRNP assembly, preventing the formation of DNA:RNA hybrids during elongation and protecting the mRNP from degradation by the exosome.

Figure 3



Mlp proteins form a selective filter at the entrance of the nuclear pore complex. **(a)** The perinuclear Mlp1p protein contributes to mRNP surveillance by retaining unspliced transcripts within the nucleus, possibly via recognition of a component associated with the 5′ splice site [57••]. **(b)** Nab2p, a shuttling mRNA binding protein involved in polyA tail length regulation, directly interacts with Mlp proteins [58•]. Nab2p may be important for the docking of mRNPs to the Mlp barrier, perhaps by signaling proper 3′ end formation. Consistent with their inessential nature, Mlp proteins may play a general role in mRNP surveillance by preferentially interacting with properly packaged mRNP complexes, preventing mRNPs that lack essential signals from reaching the central channel of the NPC.

studies establish genetic and biochemical links between Dbp5p, a shuttling DEAD-box ATPase RNA helicase essential for mRNA export, and factors involved in transcription initiation [54], pointing to a role for Dbp5p in mRNP assembly at early stages of transcription. Interestingly, mutations that impair transcription suppress *dbp5* mutant phenotypes. These observations suggest that slowing transcription may alleviate mRNP assembly defects by providing more time for proper mRNP folding. This communication between transcription and nascent mRNP formation may be viewed as another aspect of mRNP quality control.

Mlp proteins contribute to mRNP surveillance at the nuclear periphery

Mlp1p and Mlp2p are filamentous proteins, homologous to hTpr, that are anchored at the nuclear basket of the NPC. Although Mlp proteins interact with mRNP components, these proteins are not required for mRNA export and were previously proposed to function in docking or surveillance of mRNA complexes at the pore [55,56]. Consistent with this view, the Nehrbass and Jacquier groups now show that Mlp1p participates in a quality control step that prevents the export of intron-containing transcripts. The data indicate that pre-mRNA retention is mediated via the 5′ splice site, but the factor directly connecting pre-mRNA to Mlp1p remains unknown [57••] (Figure 3). Unspliced pre-mRNAs may not be the only faulty transcripts retained by Mlp proteins. Indeed, genetic interactions functionally relate Yra1p and Mlp proteins, and evidence suggests that Mlp1p and Mlp2p retain and induce a decrease in mRNA levels in a *yra1* mutant strain (Vinciguerra *et al.*, unpublished). Our current view is that Mlp proteins function as a sorting filter preferentially interacting with properly assembled mRNP particles. The inability of faulty mRNPs to dock at the Mlp barrier may negatively impact on their synthesis or stability. So far, Nab2p is the only mRNA binding protein known to directly interact with Mlp proteins [58•]. This interaction and the proposed role of Nab2p in polyA tail length regulation suggests that this protein plays a role in the docking of mRNPs to the Mlp platform, perhaps by signaling proper 3′ end formation (Figure 3).

Conclusions

mRNA export relies on a complex network of interactions that functionally couple early mRNP assembly and processing to the conserved nuclear export machinery. Recent findings reveal that transcription and export may be linked via several adaptor complexes sequentially recruited to the nascent mRNP during transcription. Whether these coupling factors contribute to the export of distinct or overlapping classes of transcripts remains to be defined. Individual components of the THO or Sus1p–Thp1p–Sac3p complexes are not essential for vegetative growth, suggesting that co-transcriptional loading of

export factors is not required under normal growth conditions, or that these recruitment pathways are partially redundant [9].

Co-transcriptional recruitment and monitoring by the exosome carrying out surveillance may favor recognition and elimination of faulty transcripts at an early step, before their release into the nucleoplasm. The proposed role of Mlp proteins in quality control reveals an additional step of mRNP surveillance at the nuclear periphery, prior to export. Interestingly, the recent study by Galy *et al*. shows that Mlp proteins localize only on sections of the nuclear envelope adjacent to chromatin [57••], suggesting that these filamentous proteins might contact active genes and exert surveillance at an early stage of mRNP formation. The physical link between SAGA and the Sus1p–Thp1p–Sac3p complex supports the view that transcriptionally active genes may indeed become tethered to the nuclear periphery [31••]. Along the same lines, the Laemmli lab has shown that tethering a genomic locus to the nuclear pore complex dramatically alters gene activity, suggesting that the NPC may more generally create an environment favorable to gene expression [59•] in addition to its newly identified function in mRNP surveillance.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Maniatis T, Reed R: **An extensive network of coupling among gene expression machines**. *Nature* 2002, **416**:499-506.

2. Neugebauer KM: **On the importance of being co-transcriptional**. *J Cell Sci* 2002, **115**:3865-3871.

3. Jensen TH, Dower K, Libri D, Rosbash M: **Early formation of mRNP: license for export or quality control?** *Mol Cell* 2003, **11**:1129-1138.

4. Bentley D: **The mRNA assembly line: transcription and processing machines in the same factory**. *Curr Opin Cell Biol* 2002, **14**:336-342.

5. Proudfoot NJ, Furger A, Dye MJ: **Integrating mRNA processing with transcription**. *Cell* 2002, **108**:501-512.

6. Kwek KY, Murphy S, Furger A, Thomas B, O'Gorman W, Kimura H, Proudfoot NJ, Akoulitchev A: **U1 snRNA associates with TFIIH and regulates transcriptional initiation**. *Nat Struct Biol* 2002, **9**:800-805.

7. Manley JL: **Nuclear coupling: RNA processing reaches back to transcription**. *Nat Struct Biol* 2002, **9**:790-791.

8. Jensen TH, Rosbash M: **Co-transcriptional monitoring of mRNP formation**. *Nat Struct Biol* 2003, **10**:10-12.

9. Stutz F, Izaurralde E: **The interplay of nuclear mRNP assembly, mRNA surveillance and export**. *Trends Cell Biol* 2003, **13**:319-327.

10. Reed R, Hurt E: **A conserved mRNA export machinery coupled to pre-mRNA splicing**. *Cell* 2002, **108**:523-531.

11. • Gatfield D, Izaurralde E: **REF1/Aly and the additional exon junction complex proteins are dispensable for nuclear mRNA export**. *J Cell Biol* 2002, **159**:579-588.
Using RNA interference in *Drosophila* cells, the authors show that REF1, as well as other components of the EJC, are not essential for mRNA export, indicating that mRNA export factors can be recruited independently of splicing.

12. Gatfield D, Le Hir H, Schmitt C, Braun IC, Kocher T, Wilm M, Izaurralde E: **The DExH/D box protein HEL/UAP56 is essential for mRNA nuclear export in *Drosophila***. *Curr Biol* 2001, **11**:1716-1721.

13. • Herold A, Teixeira L, Izaurralde E: **Genome-wide analysis of nuclear mRNA export pathways in *Drosophila***. *EMBO J* 2003, **22**:2472-2483.
This is the first global study of mRNA export pathways in higher eukaryotes. Using RNAi and DNA micro-arrays, the authors show that the export factors NXF1, NXT1 and UAP56 in *Drosophila* act in the same pathway and are essential for the export of most transcripts.

14. • Longman D, Johnstone IL, Caceres JF: **The Ref/Aly proteins are dispensable for mRNA export and development in *Caenorhabditis elegans***. *RNA* 2003, **9**:881-891.
Using RNAi, the authors show that REF proteins are dispensable for mRNA export in *C. elegans*, suggesting the existence of additional mRNA export adaptors in this organism.

15. Huang Y, Gattoni R, Stevenin J, Steitz JA: **SR splicing factors serve as adapter proteins for TAP-dependent mRNA export**. *Mol Cell* 2003, **11**:837-843.

16. • Hieronymus H, Silver PA: **Genome-wide analysis of RNA-protein interactions illustrates specificity of the mRNA export machinery**. *Nat Genet* 2003, **33**:155-161.
This genome-wide analysis shows that only a small fraction of yeast transcripts binds Yra1p, Mex67p or both, suggesting the existence of additional mRNA export factors. Evidence suggests that some export factors preferentially associate with co-regulated transcripts, perhaps through interaction with the transcriptional machinery.

17. •• Gilbert W, Guthrie C: **The Glc7p nuclear phosphatase promotes mRNA export by facilitating association of Mex67p with mRNA**. *Mol Cell* 2004, **13**:201-212.
This study shows that the yeast shuttling SR-like protein Npl3p directly interacts with Mex67p and that this association requires Npl3p dephosphorylation. The data provide evidence that dephosphorylation of Npl3p and perhaps other factors by the phosphatase Glc7p, participates in mRNA export regulation by controlling the recruitment of Mex67p to mRNP complexes.

18. Le Hir H, Gatfield D, Izaurralde E, Moore MJ: **The spliceosome deposits multiple proteins 20–24 nucleotides upstream of mRNA exon–exon junctions**. *EMBO J* 2001, **19**:6860-6869.

19. Reed R: **Coupling transcription, splicing and mRNA export**. *Curr Opin Cell Biol* 2003, **15**:326-331.

20. Linder P, Stutz F: **mRNA export: travelling with DEAD box proteins**. *Curr Biol* 2001, **11**:R961-963.

21. • Wiegand HL, Lu S, Cullen BR: **Exon junction complexes mediate the enhancing effect of splicing on mRNA expression**. *Proc Natl Acad Sci USA* 2003, **100**:11327-11332.
This study uses artificial tethering of EJC components to non-intron containing transcripts to show that the EJC, deposited normally on mRNA as a result of splicing, enhances 3′-end processing/polyadenylation and translation. The data show that the EJC is primarily responsible for the positive effect of splicing on gene expression in mammalian cells, by stimulating translation rather than mRNA export.

22. • Nott A, Le Hir H, Moore MJ: **Splicing enhances translation in mammalian cells: an additional function of the exon junction complex**. *Genes Dev* 2004, **18**:210-222.
Using artificial tethering of EJC components to reporter transcripts, this study shows that the EJC, deposited on mRNA as a result of splicing, promotes mRNA polysome association and stimulates translation.

23. Lei EP, Krebber H, Silver PA: **Messenger RNAs are recruited for nuclear export during transcription**. *Genes Dev* 2001, **15**:1771-1782.

24. Chavez S, Beilharz T, Rondon AG, Erdjument-Bromage H, Tempst P, Svejstrup JQ, Lithgow T, Aguilera A: **A protein complex containing Tho2, Hpr1, Mft1 and a novel protein, Thp2,**

connects transcription elongation with mitotic recombination in *Saccharomyces cerevisiae*. *Embo J* 2000, **19**:5824-5834.

25. Libri D, Dower K, Boulay J, Thomsen R, Rosbash M, Jensen TH:
• **Interactions between mRNA export commitment, 3′-end quality control, and nuclear degradation**. *Mol Cell Biol* 2002, **22**:8254-8266.
This paper describes genetic interactions between TREX and the nuclear exosome. Mutations in TREX result in low levels of 3′-end-truncated HSP104 transcripts retained in nuclear foci. The data show that the exosome component Rrp6p is implicated in the sequestration and degradation of incorrectly assembled mRNP complexes.

26. Strasser K, Masuda S, Mason P, Pfannstiel J, Oppizzi M,
•• Rodriguez-Navarro S, Rondon AG, Aguilera A, Struhl K, Reed R *et al.*: **TREX is a conserved complex coupling transcription with messenger RNA export**. *Nature* 2002, **417**:304-308.
This study identifies TREX, a new complex in yeast and mammals. TREX contains proteins implicated in transcription elongation (THO complex) mRNA export factors (Yra1p, Sub2p) and Tex1p, a protein of unknown function. TREX may facilitate the co-transcriptional loading of mRNA export factors.

27. Zenklusen D, Vinciguerra P, Wyss JC, Stutz F: **Stable mRNP**
• **formation and export require cotranscriptional recruitment of the mRNA export factors Yra1p and Sub2p by Hpr1p**. *Mol Cell Biol* 2002, **22**:8241-8253.
This study identifies genetic interactions between Yra1p and Sub2p, THO and the nuclear exosome. It shows that the THO component Hpr1p directly interacts with Sub2p and facilitates its co-transcriptional recruitment. In addition, transcripts assembled in a *yra1* mutant accumulate in nuclear foci and are degraded by the exosome.

28. Fischer T, Strasser K, Racz A, Rodriguez-Navarro S, Oppizzi M,
• Ihrig P, Lechner J, Hurt E: **The mRNA export machinery requires the novel Sac3p–Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores**. *Embo J* 2002, **21**:5843-5852.
The authors describe a novel complex Thp1p–Sac3p, implicated in mRNA export, and show that Sac3p interacts with Mex67p and nucleoporins. Thp1p–Sac3p may be recruited to mRNPs at an early stage and subsequently function in docking mRNP complexes to the nuclear face of the NPC via interaction with Mex67p and FG-nucleoporins.

29. Gallardo M, Aguilera A: **A new hyperrecombination mutation identifies a novel yeast gene, THP1, connecting transcription elongation with mitotic recombination**. *Genetics* 2001, **157**:79-89.

30. Lei EP, Stern CA, Fahrenkrog B, Krebber H, Moy TI, Aebi U, Silver PA: **Sac3 is an mRNA export factor that localizes to cytoplasmic fibrils of nuclear pore complex**. *Mol Biol Cell* 2003, **14**:836-847.

31. Rodriguez-Navarro S, Fischer T, Luo MJ, Antunez O,
•• Perez-Ortin JE, Reed R, Hurt E: **Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery**. *Cell* 2004, **116**:75-86.
This study identified Sus1p, which interacts with SAGA, a complex implicated in transcription initiation, and suggests that Sus1p connects SAGA to Thp1–Sac3p, an NPC-associated complex involved in mRNA export. Sac3p may be regulated by SAGA-dependent acetylation.

32. Lee TI, Causton HC, Holstege FC, Shen WC, Hannett N, Jennings EG, Winston F, Green MR, Young RA: **Redundant roles for the TFIID and SAGA complexes in global transcription**. *Nature* 2000, **405**:701-704.

33. Saleh A, Collart M, Martens JA, Genereaux J, Allard S, Cote J, Brandl CJ: **TOM1p, a yeast hect-domain protein which mediates transcriptional regulation through the ADA/SAGA coactivator complexes**. *J Mol Biol* 1998, **282**:933-946.

34. Duncan K, Umen JG, Guthrie C: **A putative ubiquitin ligase required for efficient mRNA export differentially affects hnRNP transport**. *Curr Biol* 2000, **10**:687-696.

35. Green DM, Marfatia KA, Crafton EB, Zhang X, Cheng X, Corbett AH: **Nab2p is required for poly(A) RNA export in *Saccharomyces cerevisiae* and is regulated by arginine methylation via Hmt1p**. *J Biol Chem* 2002, **277**:7752-7760.

36. Gallardo M, Luna R, Erdjument-Bromage H, Tempst P, Aguilera A: **Nab2p and the Thp1p–Sac3p complex functionally interact at the interface between transcription and mRNA metabolism**. *J Biol Chem* 2003, **278**:24225-24232.

37. Rodriguez MS, Gwizdek C, Haguenauer-Tsapis R, Dargemont C: **The HECT ubiquitin ligase Rsp5p is required for proper nuclear export of mRNA in *Saccharomyces cerevisiae***. *Traffic* 2003, **4**:566-575.

38. Neumann S, Petfalski E, Brugger B, Grosshans H, Wieland F, Tollervey D, Hurt E: **Formation and nuclear export of tRNA, rRNA and mRNA is regulated by the ubiquitin ligase Rsp5p**. *EMBO Rep* 2003, **4**:1156-1162.

39. Gilbert W, Siebel CW, Guthrie C: **Phosphorylation by Sky1p promotes Npl3p shuttling and mRNA dissociation**. *RNA* 2001, **7**:302-313.

40. Izaurralde E: **Directing mRNA export**. *Nat Struct Mol Biol* 2004, **11**:210-212.

41. Hilleren P, McCarthy T, Rosbash M, Parker R, Jensen TH: **Quality control of mRNA 3′-end processing is linked to the nuclear exosome**. *Nature* 2001, **413**:538-542.

42. Brodsky AS, Silver PA: **Pre-mRNA processing factors are required for nuclear export**. *Rna* 2000, **6**:1737-1749.

43. Hammell CM, Gross S, Zenklusen D, Heath CV, Stutz F, Moore C,
• Cole CN: **Coupling of termination, 3′ processing, and mRNA export**. *Mol Cell Biol* 2002, **22**:6441-6457.
Using yeast mutants, these authors show that 3' end cleavage, polyadenylation, transcription termination and export are tightly linked. The similarity of phenotypes of mRNA export mutants and 3' processing mutants indicates that some factors from each process may mechanistically interact to couple mRNA processing and export.

44. Dower K, Rosbash M: **T7 RNA polymerase-directed transcripts**
• **are processed in yeast and link 3′ end formation to mRNA nuclear export**. *RNA* 2002, **8**:686-697.
The authors study the export of a transcript generated by T7 RNA polymerase in yeast and show that transcription by RNA polymerase II is not required for nuclear exit, but that 3′ end processing is both necessary and sufficient for mRNA release and export.

45. Hector RE, Nykamp KR, Dheur S, Anderson JT, Non PJ,
• Urbinati CR, Wilson SM, Minvielle-Sebastia L, Swanson MS: **Dual requirement for yeast hnRNP Nab2p in mRNA poly(A) tail length control and nuclear export**. *EMBO J* 2002, **21**:1800-1810.
This paper looks at the role of Nab2p in polyA tail length regulation *in vivo* and *in vitro*, and suggests that Nab2p links termination of polyadenylation to export.

46. Marfatia KA, Crafton EB, Green DM, Corbett AH: **Domain analysis of the *Saccharomyces cerevisiae* heterogeneous nuclear ribonucleoprotein, Nab2p. Dissecting the requirements for Nab2p-facilitated poly(A) RNA export**. *J Biol Chem* 2003, **278**:6731-6740.

47. Thomsen R, Libri D, Boulay J, Rosbash M, Jensen TH: **Localization**
• **of nuclear retained mRNAs in *Saccharomyces cerevisiae***. *RNA* 2003, **9**:1049-1057.
In situ hybridization is used to localize nuclear retained HSP104 transcripts with respect to different tagged genomic loci. The data show that HSP104 nuclear foci formed in mRNA export mutants are at or close to the transcription site.

48. Andrulis ED, Werner J, Nazarian A, Erdjument-Bromage H,
•• Tempst P, Lis JT: **The RNA processing exosome is linked to elongating RNA polymerase II in *Drosophila***. *Nature* 2002, **420**:837-841.
Chromatin immunoprecipitation experiments show that the nuclear exosome is recruited to actively transcribed genes. The exosome physically interacts with RNA polymerase II and the elongation factors Spt5 and Spt6 and retains its activity when engaged in these interactions. The data suggest that the exosome monitors and degrades mRNA co-transcriptionally.

49. Chavez S, Garcia-Rubio M, Prado F, Aguilera A: **Hpr1 is preferentially required for transcription of either long or G+C-rich DNA sequences in *Saccharomyces cerevisiae***. *Mol Cell Biol* 2001, **21**:7054-7064.

50. Chavez S, Aguilera A: **The yeast HPR1 gene has a functional role in transcriptional elongation that uncovers a novel source of genome instability**. *Genes Dev* 1997, **11**:3459-3470.

51. Huertas P, Aguilera A: **Cotranscriptionally formed DNA:RNA**
•• **hybrids mediate transcription elongation impairment and**

transcription-associated recombination. *Mol Cell* 2003, **12**:711-721.
This paper indicates that mRNAs formed in the absence of THO tend to form DNA:RNA hybrids, creating an obstacle for the next polymerase and resulting in an elongation block. Co-transcriptional self-cleavage of the nascent mRNA by a hammerhead ribozyme and over-expression of RNAse H1 eliminate DNA:RNA hybrids as well as the elongation and hyper-recombination phenotypes. THO/TREX may prevent the formation of DNA:RNA hybrids during elongation by promoting efficient mRNP assembly.

52. Aguilera A: **The connection between transcription and genomic instability**. *EMBO J* 2002, **21**:195-201.

53. Jimeno S, Rondon AG, Luna R, Aguilera A: **The yeast THO**
• **complex and mRNA export factors link RNA metabolism with transcription and genome instability**. *EMBO J* 2002, **21**:3526-3535.
mRNA export mutants including *sub2*, *yra1*, *mex67* and *mtr2* show similar transcription elongation defects and hyper-recombination phenotypes as THO mutants. These results indicate a tight link between elongation and early mRNP assembly mediated by THO and mRNA export proteins, which also influences genome stability.

54. Estruch F, Cole CN: **An early function during transcription for the yeast mRNA export factor Dbp5p/Rat8p suggested by its genetic and physical interactions with transcription factor IIH components**. *Mol Biol Cell* 2003, **14**:1664-1676.

55. Kosova B, Pante N, Rollenhagen C, Podtelejnikov A, Mann M, Aebi U, Hurt E: **Mlp2p, a component of nuclear pore attached intranuclear filaments, associates with nic96p**. *J Biol Chem* 2000, **275**:343-350.

56. Strambio-de-Castillia C, Blobel G, Rout MP: **Proteins connecting the nuclear pore complex with the nuclear interior**. *J Cell Biol* 1999, **144**:839-855.

57. Galy V, Gadal O, Fromont-Racine M, Romano A, Jacquier A,
•• Nehrbass U: **Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1**. *Cell* 2004, **116**:63-73.
In this paper, genetic, biochemical and *in situ* hybridization experiments show that Mlp1p contributes to mRNP surveillance by specifically retaining unspliced pre-mRNAs within the nucleus. The study shows that Mlp proteins are asymmetrically distributed around the nuclear envelope.

58. Green DM, Johnson CP, Hagan H, Corbett AH: **The C-terminal**
• **domain of myosin-like protein 1 (Mlp1p) is a docking site for heterogeneous nuclear ribonucleoproteins that are required for mRNA export**. *Proc Natl Acad Sci USA* 2003, **100**:1010-1015.
This paper shows that Nab2p directly interacts with the C-terminal domain of Mlp1p and that over-expression of Mlp1p blocks both polyA+ and Nab2p export, suggesting it may function as a docking site for Nab2p-associated mRNA.

59. Ishii K, Arib G, Lin C, Van Houwe G, Laemmli UK: **Chromatin**
• **boundaries in budding yeast: the nuclear pore connection**. *Cell* 2002, **109**:551-562.
This paper shows that nuclear transport receptors artificially tethered to a genomic locus have boundary activity (BA), i.e. they establish non-silenced domains. BA depends on the nucleoporin Nup2p, which anchors the transport receptor to the NPC. The data indicate that physical tethering of genomic loci to the NPC can dramatically alter gene activity.