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Review Article

MAJOR PLAYERS OF RNA TRANSPORT IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT
Eukaryotic cells massively exchange macromolecules (proteins and RNAs) between the nucleus and cytoplasm through the nuclear pore complexes. Whereas a mechanistic picture emerges of how proteins are imported into and exported from the nucleus, less is known about nuclear exit of the different classes of RNAs. However, the yeast *Saccharomyces cerevisiae* offers an experimental system to study nuclear RNA export in vivo and thus to genetically dissect the different RNA export machineries. In this review, our current knowledge and recent progresses in identifying components involved in nuclear RNA export in yeast are summarized.

Keywords: *mRNA Export, SR Proteins, DBP5, Splicing, TREX, Adaptor*

INTRODUCTION
Unlike prokaryotes, eukaryotic cells segregate the vast majority of their RNA and protein synthesis into two distinct cellular compartments, i.e. the nucleus and the cytoplasm. This division necessitates nucleocytoplasmic transport pathways that can rapidly and specifically transport newly made macromolecules from their site of synthesis to their site of use. In addition, this subdivision also implies the existence of communication systems that can allow the nucleus and cytoplasm to respond in concert to changes affecting the cell. It has, in fact, now become clear that regulated changes in the sub cellular localization of specific proteins form a key component of the cellular response to the activation of many signaling pathways (Hood & Silver, 1999).

Although the nucleocytoplasmic transport of proteins and RNAs share many features in common, and indeed all nuclear RNA export is protein mediated, here I nevertheless focus exclusively on the subset of nuclear export pathways used by different classes of RNA molecule. Although I deal primarily with nuclear RNA export in metazoan cells, it is clear that these export pathways are highly conserved among eukaryotes and I will therefore, also rely extensively on data obtained in the yeast Saccharomyces cerevisiae, which has proven to be a powerful genetic system to identify critical components of several nuclear RNA export pathways. Readers interested in the mechanisms underlying protein nuclear export and, particularly, import are directed to reviews in this area (Gorlich & Kutay, 1999; Nakielny & Dreyfuss, 1999).

Coupling of Splicing to Messenger RNA Transport
The notion that splicing is linked to mRNA export first came from studies in metazoans showing that spliced mRNAs are assembled into a distinct 'spliced mRNP' complex that targets the mRNA for export (for a review, see (Reed & Hurt, 2002)). This targeting involves the splicing-dependent recruitment of the mRNA export factor Aly via its direct interactions with the spliceosomal protein UAP56. In *Saccharomyces cerevisiae*, the counterparts of Aly and UAP56 also interact with each other directly and are required for mRNA export. In both *S. cerevisiae* and metazoans, Aly also interacts directly with Tap, which, together with its binding partner p15, associates with the NPC and is thought to be a general mRNA export receptor.

These and other studies indicate that there is a conserved machinery for mRNA export, (for reviews, see (Dreyfuss *et al.*, 2002; Izaurralde, 2002; Lei & Silver, 2002; Reed & Hurt, 2002). In metazoans, where most genes contain introns, this conserved machinery is intimately tied to the splicing machinery. By contrast, in *S. cerevisiae*, where few genes contain introns, it appears that mRNA export is coupled to other steps in gene expression.

An Important Role of SR Proteins in mRNA Export

Although this review has focused on the conserved TREX (transcription/export) export machinery, proposals have been made that there are multiple physiologically relevant adaptors for mRNA export distinct from TREX (Gatfield & Izaurralde, 2002). 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 (Gatfield *et al.*, 2001; Rehwinkel *et al.*, 2004). Although other adaptors have been proposed, the only general candidates so far reported are members of the SR family of splicing factors (Huang & Steitz, 2001). 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 (Fu, 1995)). After splicing, SR proteins remain bound to the spliced mRNA (for review, see Graveley, 2000). SR proteins also shuttle between the nucleus and cytoplasm, as would be expected of an mRNA export protein. More recent studies showed that SR proteins mediate mRNA export (Huang *et al.*, 2003; Huang & Steitz, 2001). Moreover, the SR proteins involved (9G8 and SF2) function by direct interaction with TAP. SR proteins are known to be serine-phosphorylated, and the phosphorylation state is regulated during splicing (Graveley, 2000). 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 (Huang *et al.*, 2003). 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.

In yeast, Npl3 is an SR-like shuttling protein that has no known role in splicing but functions in mRNA export. This protein is co-transcriptionally recruited to active genes and interacts with RNAP II (Lei *et al.*, 2001). 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. 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 (Gilbert & Guthrie, 2004). 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 proteins that do associate with the TREX complex and require THO complex components for export (Hacker & Krebber, 2004; Hurt *et al.*, 2004). ChIP and RNA immune precipitation studies show that these proteins are associated with actively transcribed genes throughout their lengths and are bound to nascent transcripts. 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, it would be interesting to determine whether there is an association between SR proteins and the mammalian TREX complex as in yeast.

Nuclear Pore Complex and Dbp5 Connection

Dbp5 is highly conserved among eukaryotes and was identified initially through both a genetic screen to isolate yeast mutants defective in mRNA export (Snay-Hodge *et al.*, 1998) and through an analysis of the localization and possible roles of a number of yeast DEAD-box proteins (Tseng *et al.*, 1998). Dbp5 is concentrated at the NPC, where it interacts directly with two nucleoporins, Nup159 (CAN/NUP214 in metazoan cells) and Gle1 (hGLE1). Dbp5 is relatively abundant and most of the non-NPC-associated fraction is located in the cytoplasm, although Dbp5 shuttles between nucleus and cytoplasm and a substantial fraction accumulates rapidly in nuclei when export mediated by exportin 1 (Xpo1 in yeast; CRM1 in metazoans) is blocked (Hodge *et al.*, 1999).

Dbp5 has ATPase activity *in vitro* (Tseng *et al.*, 1998) and mutations that eliminate this activity renders the protein non-functional *in vivo* (Schmitt *et al.*, 1999). DEAD box proteins are sometimes called RNA helicases because several are able to unwind short regions of double-stranded RNA *in vitro* (for review,

see (Rocak & Linder, 2004)). In two studies, helicase/unwinding activity of yeast and human Dbp5 was detected, but only if an extract was added to purified Dbp5, indicating the need for co-factors.

Important for mRNA export is the recent finding that some DEAD-box proteins are able to remove stably bound proteins from RNA (Fairman *et al.,* 2004; Jankowsky *et al.,* 2001). We know that some mRNP proteins are exported with the mRNA and subsequently removed from mRNPs as they arrive in the cytoplasm, so it is reasonable to hypothesize that this is a key function performed by Dbp5.

RNA Export is Linked to 3' End Processing and Modification

Capping, splicing and poly (A) tail formation are all known to increase efficiency of mRNA export. Each step is marked by the deposition of specific factors which better allow transport factors to associate with the RNA (Hocine *et al.,* 2010; Katahira, 2015). However, there are ways around these processing events. For instance, while splicing is important for export, intron-retained RNAs can be exported (Li *et al.,* 2006; Wang *et al.,* 2015). Normally, export of unspliced or intron-retained RNAs is blocked at the nuclear basket by TPR. Indeed, the inactivation of TPR leads to an increase in export of intron-retained mRNAs (Coyle *et al.,* 2011). Export of intron-retained transcripts leads to translation leading to proteins with different functionalities. Classically, viruses export unspliced RNAs for packaging into virions, e.g., the RRE (Rev response element) in HIV binds the viral protein REV which is then exported by CRM1 by virtue of its NES (Ernst *et al.,* 1997). However, human mRNAs can also be exported with introns. For example, intron retained forms of NXF1 mRNA itself are exported by the NXF1 protein (Li *et al.,* 2006; Wang *et al.,* 2015).

Another question that arises given the relevance of splicing to export is how transcripts without introns are efficiently exported given they do not associate with the EJC. Interestingly there have been multiple export pathways which allow export of such transcripts. For instance, many intronless mRNAs contain a specific element in the 3′ UTR known as cytoplasmic accumulation regions (CAR) (Lei *et al.,* 2011). Finally recent studies indicate that there are other covalent modifications can modulates RNA fate. For example, N6-methylation of RNAs can modulate mRNA export, as well as translation efficiency and stability. This process is reversible suggesting that even fully matured mRNAs could have their export modulated by these modifications (Fu *et al.,* 2014).

Role of Mlp Proteins to mRNP Surveillance

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 (Kosova *et al.,* 2000; Strambio-De-Castillia *et al.,* 1999). 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 (Galy *et al.,* 2004). 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. The 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 (Green *et al.,* 2003). 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.

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.
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.* (2004) showed that Mlp proteins localize only on sections of the nuclear envelope adjacent to chromatin, 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. 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 in addition to its newly identified function in mRNP surveillance.

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