REVIEW

Nuclear RNA export

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RNA export is the process by which RNAs are transported to the cytoplasm after synthesis, processing, and RNP assembly within the nucleus. The primary focus of this review is mRNA export with particular attention paid to the yeast *Saccharomyces cerevisiae*. Because there is rather little known about mRNA export in general and even less about yeast mRNA export, our thinking about the problem is influenced by information from other transport processes. These include not only mRNA export in vertebrate systems but also studies on the export of other RNA substrates and even studies on protein import. Several of these areas of investigation have recently intersected in gratifying ways.

Distinct export pathways for the different classes of RNAs

The first area of investigation to be discussed is the study of RNA export in the *Xenopus* oocyte system. This approach relies largely on injection experiments, in which different RNA substrates are injected into *Xenopus* germinal vesicles (nuclei) and cytoplasmic export assayed as a function of time after injection. Pioneering experiments of this kind indicated that the general process of nuclear export is temperature dependent and saturable (Zasloff 1983). Coinjection experiments indicated that different RNA substrates use nonidentical and perhaps entirely different pathways. For example, experiments showed that tRNA, RNA polymerase II gene products (mRNAs and U snRNAs), and ribosomal subunits all used saturable pathways, but any one substrate did not saturate the export of another (Bataille et al. 1990; Jarmolowski et al. 1994; Pokrywka and Goldfarb 1995). Further experiments indicate that U snRNAs also access an export pathway that is shared with both 5S RNA and proteins that carry a specific type of nuclear export signal (NES, see below; Fischer et al. 1995). In higher eukaryotic cells, U snRNAs are exported shortly after synthesis. They pick up snRNP proteins and undergo processing events in the cytoplasm, prior to reimport back into the nucleus (Fischer and Lührmann 1990; Hamm et al. 1990a; Fischer et al. 1993). (Export and processing of U snRNAs in yeast cytoplasm has not been demonstrated yet.)

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The HIV-1 Rev protein: a prototype RNA export factor

The second area described is the study of regulated RNA export in viral systems and the function of the HIV-1 protein Rev. There is voluminous literature on this subject, which has also been reviewed extensively (e.g., Cullen and Malim 1991). However, Rev has been critical to our current mechanistic understanding of export and a few words of introduction are unavoidable. During the viral life cycle, Rev contributes to the switch from early to late gene expression. In the early phase, products from fully spliced RNAs, including Rev, are synthesized. HIV-1 splicing is inefficient, however, and unspliced as well as partially spliced transcripts accumulate within the nucleus. In the late phase, Rev function results in the appearance of these viral transcripts in the cytoplasm, allowing the production of the viral structural proteins Gag, Pol, and Env, as well as the packaging of genomic RNAs into viral particles (Feinberg et al. 1986; Sodroski et al. 1986). Reporter gene experiments in tissue culture cells showed that Rev functions to promote the expression of unspliced RNA (Malim et al. 1989a). They also identified a region of the *env* pre-mRNA that is necessary for Rev function. Rev is an RNA-binding protein and directly recognizes this RNA motif, the RRE (Rev response element; Hadzopoulou-Cladaras et al. 1989; Malim et al. 1989). This interaction gives specificity to Rev and explains how it selects *env* pre-mRNA for export to the cytoplasm (Heaphy et al. 1990; Malim et al. 1990; Zapp and Green 1989). As Rev increases the expression of RRE-containing RNAs even in yeast (Stutz and Rosbash 1994), Rev function and the cellular proteins with which it interacts must be highly conserved.

The most straightforward explanation of Rev's function is to directly export pre-mRNA export, as first proposed some time ago (Felber et al. 1989; Malim and Cullen 1993). A second possibility is that Rev directly inhibits pre-mRNA splicing (Chang and Sharp 1989; Kjems et al. 1991). The positive effects on export would then be indirect; splicing inhibition would lead to an increase in pre-mRNA levels, which would then lead to an increase in export through the normal cellular RNA route. Although there is some evidence that Rev has negative effects on splicing (Kjems and Sharp 1993), the compelling nature of subsequent experiments argues that the Rev effect is predominantly to promote pre-mRNA export.

This export function requires Rev's second functional domain, which is carboxy-terminal to the region that interacts with RNA and which contains several critically spaced leucine residues. Early mutagenesis experiments, largely by Cullen and coworkers, showed that this effector domain or NES was critical for Rev function (Malim et al. 1989a, 1991). Several papers then indicated that export was the dominant focus of this leucine-rich domain. Lührmann and colleagues showed that Rev was able to promote nuclear export of RRE-containing RNAs in *Xenopus* oocytes independent of the presence of introns, indicating that splicing was probably unnecessary for Rev function (Fischer et al. 1994). Export was also indicated by yeast two-hybrid experiments, which identified novel yeast and mammalian nucleoporins (or nucleoporin-like proteins) as candidate Rev NES-interacting proteins with functional effects on Rev-mediated export. These proteins, including the yeast protein Rip1p/Nup42p, were proposed to be export receptors within the nucleus or docking sites on the nuclear side of the pore (Bogerd et al. 1995; Fritz et al. 1995; Stutz et al. 1995).

A contemporary study showed that a peptide comprising the Rev NES could specifically inhibit Rev-mediated export. As this paper showed that bovine serum albumin (BSA) coupled to the Rev peptide (BSA-R) was exported from the nucleus in mammalian cells as well as in oocytes, RNA export could be viewed as a protein export process with associated RNA cargo (Fischer et al. 1995). This shift in perspective was reinforced by a study showing that a cellular factor, the protein kinase A inhibitor PKI, contains a functional Rev-like NES (Wen et al. 1995). There was also evidence for a connection between Rev-mediated export and cellular RNA export. U snRNA and 5S RNA export, but not other RNA export pathways, was inhibited by injection of BSA-R into oocytes; no inhibition was observed with BSA-M, which contained the export-deficient M10 mutant NES (Malim et al. 1989a). This finding established a tight connection between the protein export pathway mediated by Rev and this particular cellular RNA export pathway (Fischer et al. 1995). It suggested further that the latter uses one or more Rev-like NES-containing proteins as RNA transporters and that the Rev and U snRNA pathways share one or more dedicated components.

Searching for these components proceeded along several seemingly logical fronts. A yeast system was established with the hope of applying genetic tools and identifying candidate Rev-interacting proteins. Although the Rev effect in yeast is sufficient for analytical purposes, unfortunately it is not strong enough to be used in genetic screens (Stutz et al. 1995). The Rev-like NES also proved to be an enigmatic sequence motif. Other than several leucines or hydrophobic residues arranged with characteristic spacing, there are few diagnostic features (Bogerd et al. 1996). This made it difficult to identify cellular NESs with search paradigms and probably indicates that its unique structural features (at least in the absence of other proteins) are quite subtle. Although several candidate Rev-binding proteins have been identified biochemically, none has been convincingly implicated in Rev-mediated transport (Ruhl et al. 1993; Bevec et al. 1996; Schatz et al. 1998). There is reason to believe that the nucleoporin-like proteins identified by the yeast two-hybrid approach are more credible interactors, probably because this in vivo assay allows for a contribution by additional relevant yeast proteins to the two-component bait and prey complex (see below).

The importin-β family of transport receptors

The third intersecting area is that of nuclear import receptors and their relatives, the importin-β family. Indeed, the basic framework for thinking about RNA and protein export comes from the protein import field. Although there is still considerable mystery surrounding the nuclear pore itself and how import substrates traverse this large and complex organelle, during the past few years there has been startling progress in our understanding of the 'soluble phases' of the protein import process (Mattaj and Englmeier 1998). These are concerned with the events that occur in the cytoplasm prior to import and in the nucleoplasm subsequent to import. In the cytoplasm, a transport complex is assembled, which then docks at the pore. After traversing the pore and arriving within the nucleus, the transport complex is disassembled, the cargo liberated, and the transport factors recycled back to the cytoplasm (for review, see Görlich and Mattaj 1996a; Görlich 1997; Nigg 1997).

Progress in understanding this soluble phase is due in part to a convergence of protein biochemistry and yeast genetics and has led to a near universal acceptance of the starting point: the cytoplasmic association of cargo with an import receptor. Different import substrates use different receptors, which are all members of the importin-β family—also called karyopherin-β (Fornerod et al. 1997b; Görlich et al. 1997; for review, see Görlich 1997, 1998; Weis 1998; Wozniak et al. 1998). In the best-studied case, nuclear localization signal (NLS)-containing substrates interact indirectly with importin-β by binding directly to the importin-α subunit (Adam and Gerace 1991; Görlich et al. 1994, 1995b; Imamoto et al. 1995; Weis et al. 1995). Other import substrates have different signals and bind directly to other importin-β family members. Not all of these importins are essential in yeast, implying a certain level of functional redundancy (for review, see Pemberton et al. 1998; Weis 1998; Wozniak et al. 1998). There are also important cofactors that assemble in a stable manner with substrate and receptor. These include the small GTPase Ran/TC4 (Gsp1p and Gsp2p in yeast; for review, see Koepp and Silver 1996; Corbett and Silver 1997; Görlich 1997; Cole and Hammell 1998; Dahlberg and Lund 1998; Izaurralde and Adam 1998). Ran may contribute to the docking of the NLS-receptor complex at the cytoplasmic face of the pore, which probably occurs through a direct interaction between the receptor and FXFG and GLFG repeat se-

quence-containing nucleoporins (Adam and Adam 1994; Görlich et al. 1995a; Iovine et al. 1995; Kraemer et al. 1995; Moroianu et al. 1995; Radu et al. 1995b; Rexach and Blobel 1995). Ran-dependent GTP hydrolysis also provides energy for the translocation through the pore, and Ran is required for the dissociation of the substrate-receptor complex on the nuclear side of the pore (Görlich et al. 1996).

The fact that Ran contributes to complex dissociation in the nucleus but allows complex formation in the cytoplasm is superficially enigmatic but fits well with other aspects of Ran's life style. Ran is inferred to be largely complexed with GDP in the cytoplasm and largely complexed with GTP in the nucleus. This presumed gradient reflects the fact that the Ran GTPase activating protein RanGAP1 (Rna1p in yeast) is primarily cytoplasmic (Hopper et al. 1990), and the Ran guanine nucleotide exchange factor RCC1 (Prp20p in yeast) is primarily nuclear (Ohtsubo et al. 1989). RanGAP1 increases the intrinsic GTPase activity of Ran by up to five orders of magnitude and stimulates the conversion of Ran–GTP into Ran–GDP. The activity of Ran–GAP1 is itself stimulated 10-fold by the mainly cytoplasmic Ran binding protein RanBP1 (Yrb1p in yeast; Bischoff et al. 1995; Schlenstedt et al. 1995; Richards et al. 1996). It makes sense, therefore, that import substrate–receptor complex formation occurs in the presence of Ran–GDP but is inhibited by Ran–GTP (Rexach and Blobel 1995; Görlich et al. 1996; Chi et al. 1997). Apparently, the reverse is true for export substrate–receptor complex formation: Ran–GTP is important if not essential for the formation of these complexes (Arts et al. 1998; Fornerod et al. 1997a; Izaurralde et al. 1997b; Kutay et al. 1997, 1998; for review, see Cole and Hammell 1998; Dahlberg and Lund 1998; Izaurralde and Adam 1998; see below).

In addition to the importin-β family members known to function in protein import, several other importin-β-like proteins were identified by database homology searches (Fornerod et al. 1997b; Görlich et al. 1997; Ullman et al. 1997; for review, see Pemberton et al. 1998). These were proposed to be novel transport factors based on two arguments. First, their conservation is highest at the amino terminus, where importin-β is known to interact with Ran (Görlich et al. 1996, 1997; Chi et al. 1997; Fornerod et al. 1997b). Second, one of the family members with no known function, human CRM1 (chromosome region maintenance 1), interacted directly with the nucleoporin CAN/Nup214 (Fornerod et al. 1997b). This resembled the direct interaction of importin-β to repeat-containing nucleoporins described above.

CRM1, the export receptor for Rev

Unexpectedly, the cytotoxin leptomycin B was the fourth intersecting area and provided a key experimental tool to identify the direct receptor for Rev-mediated export. Leptomycin B had been previously shown to kill *Schizosaccharomyces pombe* and its apparent molecular target was the product of the gene *CRM1*; leptomycin B-resistant mutants of *S. pombe* map within the *CRM1* gene (Nishi et al. 1994). Importantly, leptomycin B was then shown to block both Rev export and Rev-dependent RNA export in mammalian cells (Wolff et al. 1997). Given that CRM1 is an importin-β family member (Fornerod et al. 1997b; Görlich et al. 1997), these data led to the exciting possibility that it might be the Rev NES receptor (Fig. 1).

Indeed, biochemical experiments in the Mattaj laboratory showed that overexpression of CRM1 in *Xenopus* oocytes stimulates both Rev and U snRNA export. Conversely, leptomycin B inhibits these two pathways, and this inhibition can be overcome by coinjection of excess CRM1. In vitro, CRM1 forms a complex with Ran–GTP and NES sequences from either Rev or PKI and the association is cooperative; in addition, leptomycin B inhibits complex formation, as the in vivo data predict (Fornerod et al. 1997a). The fact that Ran–GTP is required for the Rev–CRM1 interaction explains the earlier lack of success in the biochemical isolation of bona fide Rev-interacting proteins. Two other groups also identified a direct interaction between CRM1 and cellular proteins with a Rev-like NES, but with no apparent requirement for Ran–GTP (Fukuda et al. 1997; Ossareh-Nazari et al. 1997). Taken together, all the results indicate that the importin-β family member CRM1 is the Rev NES export receptor. The cooperative nature of the tripartite interaction probably explains why biochemical fishing experiments with NES have been so unsuccessful: The absence of high levels of Ran–GTP in total extracts precludes a strong interaction between Rev NES and export receptors. The yeast two-hybrid experiments, on the

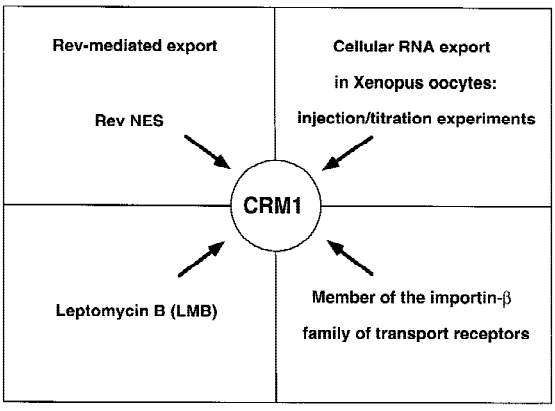

Figure 1. CRM1 is the export factor for Rev-like NES containing substrates. Independent observations converged at the identification of CRM1. *S. pombe* strains resistant to the cytotoxin leptomycin B had been mapped to the *CRM1* gene. *Xenopus* oocyte injections showed that Rev uses a cellular export pathway dedicated to U snRNAs and 5S RNA; this pathway is inhibited by leptomycin B. CRM1 was identified in a complex with the FG–nucleoporin CAN/Nup214 and shown to be a member of the family of transport factors related to importin-β. Rev NES directly interacts with CRM1 and this interaction is sensitive to leptomycin B (see text for references).

contrary, benefitted from the high levels of Ran–GTP present within yeast nuclei.

Two other papers addressed the role of CRM1 in yeast nuclei, that is, the role of the *S. cerevisiae* ortholog Crm1p. One created and utilized a sensitive NLS–GFP–NES reporter protein (Stade et al. 1997). In wild-type cells, it is localized predominantly in the cytoplasm, indicating that NES activity dominates the phenotype. A temperature-sensitive (ts) *CRM1* mutant (also called *xpo1-1*) led to relocalization of the reporter protein to the nucleus. The inference is that the loss-of-function mutation decreased NES activity and therefore increased the ratio of NLS:NES activities. Crm1p was also shown to shuttle between the nucleus and cytoplasm. Taken together with a two-hybrid interaction between the Rev NES and Crm1p, the data were consistent with the notion that Crm1p is the Rev NES export receptor in yeast (Stade et al. 1997). The second utilized three viable loss-of-function mutations in *CRM1* (Neville et al. 1997). These mutations were originally identified based on their effects on localization of the yeast transcription factor Yap1p. Yap1p has a Rev-like NES and its nuclear export is regulated (C. Yan. L.H. Lee, L.I. Davis, in prep.). All three mutations led to a significant decrease in Rev NES-mediated RNA export. Taken together with a NES-Crm1p two-hybrid interaction as well as a strong interaction between Crm1p and Rip1p/Nup42p, these results also suggested that Crm1p was the export factor for the Rev NES. The three mutations also affected the two-hybrid interaction between a NES and Rip1p/Nup42p (Neville et al. 1997). As this is the interaction that originally identified the candidate nucleoporin Rip1p (Stutz et al. 1995), it suggests that NES–nucleoporin interactions might be indirect and mediated by Crm1p. This is precisely the role one imagines for an export receptor, namely, delivery of the export cargo to the nuclear pore. Consequently, the data from *Xenopus* oocytes, mammalian cells, and *S. cerevisiae* lead to a strong conclusion: Crm1p is the export receptor for Rev NES-containing proteins.

Export receptors for tRNA and importin-α

Two other importin-β family members have also been assigned roles in export. The protein CAS is the exporter for the importin-α subunit. Similar to the requirements for complex formation between CRM1 and its substrate Rev NES, an in vitro interaction between CAS and importin-α requires Ran–GTP. CAS shuttles and binds preferentially to NLS-free importin-α, which might aid in nuclear dissociation of importin-α from its substrates and also presumably decreases the capacity of importin-α to reexport import substrates (Kutay et al. 1997).

The third importin-β family member involved in export is called exportin-t because it exports tRNA from the nucleus. In contrast to the earlier comment about RNA export being a protein export problem, this member of the importin-β family binds and exports RNA rather than protein. Like the two other exportins, exportin-t shuttles and binds its substrate well only in the presence of Ran–GTP (Arts et al. 1998; Kutay et al. 1998). Indeed, the data support a cooperative interaction between exportin-t, Ran–GTP, and tRNA substrate. In permeabilized mammalian cells, addition of Ran–GTP causes a relocalization of recombinant exportin-t from the nucleoplasm to nuclear pores, consistent with the binding of exportin-t complexes to pores. Injection experiments in the oocyte system showed that exportin-t stimulates the rate or levels of tRNA export and overcomes the saturating effect of additional tRNA. The nature of the tRNA–exportin-t interaction will be interesting because the protein has no known RNA binding motif and because there is evidence that tRNA modifications as well as a mature 3′CCA end contribute to binding specificity (Arts et al. 1998; Kutay et al. 1998). A preference for processed tRNA is consistent with the lack of evidence for an exportin-t role in processing; perhaps exportin-t binds its substrate after most processing has taken place.

These results raise interesting questions when they are compared with data from *S. cerevisiae*. Although only distantly related to exportin-t, the importin-β family member Los1p appears to be the yeast ortholog, and recent in situ hybridization experiments indicate nuclear accumulation of tRNA in *LOS1* loss-of-function mutants (Sarkar and Hopper 1998). Los1p has been known for almost 20 years to be important for yeast tRNA processing and the original *los1-1* mutation causes inefficient tRNA splicing (Hopper et al. 1980; Hurt et al. 1987). Also, Los1p interacts with nuclear pore complexes, consistent with the general exportin picture (Simos et al. 1996). However, the gene is inessential, indicating that there must be an additional pathway for tRNA export. These genetic data are consistent with in situ hybridization data showing that the nuclear tRNA signal in *LOS1* mutants is more modest than in some nucleoporin mutants, such as *NUP116* temperature-sensitive mutants (Sarkar and Hopper 1998) or in Ran cycle mutants (H. Grosshans, E. Hurt, and G. Simos, unpubl.). In considering a relationship to processing, it is worth remembering that the tRNA splicing enzymes, endonuclease and ligase, are associated with the nuclear membrane (Abelson et al. 1998). As *LOS1* is inessential, Los1p probably 'helps' deliver the pre-tRNA substrate to these activities, that is, to the nuclear membrane. But this more relaxed view is somewhat at odds with a stable nuclear exportin-substrate complex that persists until it reaches the cytoplasmic face of the pore. Perhaps the nuclear exportin-t complex is more dynamic in vivo and engages in multiple cycles of association and disassociation. Alternatively, pre-tRNA splicing might take place while the substrate is bound within the exportin-t complex.

Does mRNA transport use an exportin?

This question has two parts: Does mRNA transport use one of the three identified exportins, and is there more

general evidence that mRNA transport uses exportins, perhaps another family member? With respect to the first part, there is no evidence that CAS or exportin-t is involved in mRNA export. But the connection between CRM1 and mRNA transport is intriguing, especially in yeast.

In this system, a connection between Crm1p and mRNA transport has been made by the *xpo1-1* mutant: After a shift to the nonpermissive temperature, mRNA export as well as Rev NES-mediated transport is rapidly and strongly inhibited (Stade et al. 1997). The simple interpretation is that Crm1p makes an important contribution to mRNA export. However, it is possible that the block to mRNA export is indirect, attributable to a more direct effect of the *CRM1* temperature sensitive mutation on another aspect of transport. The likelihood that this is protein import is somewhat reduced by the fact that these investigators examined this mode: It was affected much more slowly than mRNA export. But there are multiple protein import pathways, and the more general concern still remains: A block of one mode of transport (NES-mediated protein export) might indirectly inhibit another (mRNA export). Consistent with the lack of a direct connection between these two modes is the viable nature of the three *CRM1* loss-of-function alleles. This suggests no more than a modest effect on mRNA export. Indeed, these viable alleles have no detectable impact on mRNA export as assayed by in situ hybridization, despite a potent effect on Rev-mediated RNA (Neville et al. 1997) as well as protein export (M. Neville and M. Rosbash, unpubl.). In higher eukaryotes, the absence of a role for CRM1 in mRNA export is also supported by the following observations: As mentioned above, injection of Rev NES into oocytes competes with U snRNA export but has no effect on mRNA export, suggesting that this pathway is distinct from the CRM1-mediated pathway shared by Rev NES and U snRNAs (Fischer et al. 1995; Fornerod et al. 1997a). More importantly, leptomycin B does not inhibit mRNA export in oocytes and mammalian cells, whereas it strongly inhibits U snRNA export (Fornerod et al. 1997a; Wolff et al. 1997). In addition, overexpression in mammalian cells of a truncated, defective form of the nucleoporin CAN/Nup214 that retains CRM1 binding ability inhibits Rev-dependent gene expression but has no effect on the cellular mRNA export pathway (Bogerd et al. 1998). However, some of these results are controversial. A recent contribution suggests that oocyte injection of higher NES peptide concentrations, conjugated with a different carrier protein, inhibits mRNA export. Because there is also an inhibitory effect with the M10 mutant peptide, previously shown to have no NES activity, it is possible that the effect is only marginally relevant to NES-mediated export (Pasquinelli et al. 1997b). At this time, one must therefore remain skeptical but open to the possibility that Crm1p and Rev-like NESs contribute to mRNA export.

Are there indications that mRNA export requires another exportin-like receptor? Oocyte injection experiments have shown that nuclear depletion of Ran–GTP inhibits the export of a variety of substrates including Rev, U snRNAs, and mRNAs (Izaurralde et al. 1997b). Injection of the GTP hydrolysis-resistant GTPase RanQ69L (in subinhibitory amounts) restores Rev and partial mRNA export but not U snRNA export, indicating different GTP hydrolysis requirements for the export of different classes of substrates. Also, different mRNAs show different behaviors with respect to this GTP–hydrolysis requirement. This might reflect the existence of different mRNA export pathways or the presence of distinct hnRNP proteins on different transcripts (Izaurralde et al. 1997b; Saavedra et al. 1997a). In any case, the strict Ran–GTP requirement observed for the export of most mRNAs tested so far is consistent with a contribution of importin-β like factor(s) to mRNA export.

What substrates for Crm1p?

If one assumes that Crm1p is not relevant to mRNA export, then one should look to other RNA pathways or to protein export for the source of Crm1p substrates. This also questions the nature and role of yeast NESs recognized by Crm1p. Presumably there are yeast proteins, like mammalian PKI, whose export is constitutive or regulated. One is the yeast protein Yap1p, which has a bona fide Rev-like NES and whose transport is regulated by Crm1p (C. Yan L.H. Lee, L.I. Davis, in prep.). The *S. pombe* homolog Pap1p is similarly regulated by Crm1p (Toone et al. 1998). Although there are several additional published proteins with some of the expected properties, (the yeast proteins Gle1p or Mex67p for example; see below), these are only candidates at present. This is due to the fact that Rev-like NES sequences are so easy to find and so difficult to define unambiguously by statistical methods. We also believe that NES–reporter gene assays are not without interpretation difficulties: A leucine-rich region might function in this context but not necessarily in its natural location. At a minimum, extensive mutagenesis of a putative NES, preferably in its natural location as first done by Cullen and colleagues on Rev (Malim et al. 1989a, 1991), is necessary to demonstrate convincingly Rev-like NES function. Note that this type of analysis on the Rev NES in yeast indicates that the Rev NES–Crm1p interaction (presumably Rev NES–Crm1p–Ran–GTP) closely resembles the Rev NES–CRM1 interaction of mammals (Stutz et al. 1995, 1996).

The current absence of many bona fide Rev NES-containing yeast proteins has an interesting and unexpected implication: Yeast NESs may not resemble perfectly a canonical Rev NES. A substantial difference would lead not only to different NES mutagenesis results with a candidate yeast NES but would also help explain the phenotypes of the three viable loss-of-function *CRM1* mutants. These mutants have strong effects on Rev but little or no effect on growth and mRNA export (Neville et al. 1997). Were the Rev NES to be an imperfect mimic of a natural yeast NES, the former would fit less well

into the Crm1p NES-binding pocket and therefore be more sensitive to mild Crm1p loss-of-function mutations. A difference between the NES-binding pockets of Crm1p and CRM1 proteins from other species is also consistent with a significant difference in leptomycin B-sensitivity. The insensitivity of *S. cerevisiae* to this reagent is not due to permeability but to an actual physical difference between yeast Crm1p and *S. pombe* Crm1p (Fornerod et al. 1997a). Yeast two-hybrid interactions between Rev NES and *S. pombe* Crm1p are leptomycin B-sensitive, whereas the comparable interaction with the *S. cerevisiae* protein is insensitive (M. Neville and M. Rosbash, unpubl.). Consequently, one must remain open to the possibility of yeast substrates that differ from a canonical Rev or PKI NES as well as to different physiological roles of Crm1p-mediated export in this organism—including mRNA export.

The most likely role of CRM1 in RNA export is still U snRNA and 5S RNA export in vertebrate systems. As described above, this is because of the impressive inhibition of U snRNA export by injection of excess Rev NES peptide or leptomycin B into oocyte nuclei. Because injection of excess mono-methyl G cap also inhibits these pathways, it was proposed that the cap connects to the export machinery that carries these RNAs out to the cytoplasm (Hamm and Mattaj 1990). This led to the purification and cloning of the nuclear cap-binding complex (CBC), CBP20 and CBP80. These proteins bind to the cap shortly after it is added to RNA on nascent transcription complexes and contribute to their nuclear export (Hamm and Mattaj 1990; Izaurralde et al. 1992, 1995). There is now evidence for an in vitro interaction between CBC and CRM1 (I. Mattaj, pers. comm.). If CBC physically and conceptually links U snRNAs to CRM1, this provides, at least in principle, a complete picture of snRNA transport from nascent transcription to the pore. Presently, it is unclear whether this description also applies to yeast U snRNAs and Crm1p. As mentioned above, it has not been shown that yeast U snRNAs are exported and reimported into nuclei as in vertebrate systems. It is possible that yeast snRNPs never leave the nucleus, which would indicate that the addition of Sm proteins, snRNP assembly, and cap trimethylation are entirely nuclear processes in this organism.

A role for CBC and possibly CRM1 in snRNA export thus raises the question of why the data so clearly show that this interaction is not essential for mRNP transport in vertebrates (Fornerod et al. 1997a; Izaurralde et al. 1995). This is despite the fact that RNA polymerase II pre-mRNA transcripts are capped and decorated by CBC indistinguishably from newly synthesized snRNA transcripts (Visa et al. 1996b). We can imagine two possibilities. First, the interaction between CBC and CRM1 may not occur or may be altered in nuclear mRNP relative to snRNP. For example, the presence of an adjacent hnRNP protein in mRNP could influence CBC and affect its interaction with CRM1. Second, mRNA could be intrinsically 'more difficult' to transport than a small structured RNA. The same cap–CBC–CRM1 complex might be sufficient or play a major role in the case of the latter but not the former. This offers an attractive explanation for the mRNA export block in the yeast *CRM1* temperature-sensitive mutant: (1) Crm1p binds to CBC and is a component of nuclear RNP, (2) this Crm1p activity aids but is insufficient alone for mRNP export, and (3) at the nonpermissive temperature, the mutant Crm1p has a negative effect on the mRNPs to which it is bound. Another explanation is that Crm1p is involved in the reexport of factors that can inhibit mRNA export. Crm1p-mediated export may normally prevent their nuclear accumulation.

Nuclear retention vs. nuclear export

Some of the above considerations suggest that successful export must be sufficiently robust to overcome RNA or RNP retention within the nucleus. As export is a selective process, failure may not only reflect the absence of a strong export signal but also the presence of a strong nuclear retention signal. Indeed, there are clear circumstances where these two processes (RNA export and RNA retention) are in competition.

This principle has been articulated in detail within the context of Rev-mediated pre-mRNA transport. In this system, spliceosome assembly inhibits pre-mRNA export presumably because one or more spliceosome components block transport. The block might reflect an intrinsic feature of mature snRNPs, which are restricted to nuclei. In this view, a simple Rev transport function is not an entirely satisfactory explanation. How does Rev overcome spliceosome-mediated retention? Consistent with a role of Rev in spliceosome disassembly is a result from the yeast system: The introduction of a branchpoint mutation into a Rev-responsive reporter pre-mRNA prevents Rev-dependent pre-mRNA export; the mutation, which blocks the first step of splicing, is epistatic to Rev's positive effect on pre-mRNA cytoplasmic localization (Stutz and Rosbash 1994). We suggest that Rev influences features of splicing, at least retention features, in addition to its effects on transport. An inference is that antiretention features, such as spliceosome disassembly factors, are concentrated at or near nuclear pores—analogous to the nuclear membrane localization of tRNA splicing factors. In this scenario, the Rev export receptor would aid in spliceosome disassembly by bringing substrates to the pores. This would serve to increase the local concentration of the relevant factors and inhibit retention. Although the branchpoint mutant would be expected to lower the efficiency of spliceosome assembly, it is conceivable that it has an even more deleterious effect on pre-mRNA spliceosome disassembly. The Rev-mediated increase in local enzyme concentration would be insufficient to disassemble the mutant spliceosomes.

An alternative, not necessarily incompatible, view relies on the propensity of Rev to polymerize along an RNA transcript. For example, the complete viral RRE element is known to favor the binding of multiple Rev molecules (Heaphy et al. 1991; Zapp et al. 1991). This

might serve two protransport functions: It would increase the number of bound CRM1 molecules and presumably CRM1 export activity, and it might inhibit retention by competing with and displacing spliceosome components or other retention elements. CRM1 could even aid in Rev polymerization (Hakata et al. 1998).

If CRM1 does not contribute directly to cellular mRNP export, other positive transport factors must exist. Some of these may even be deposited on the RNA substrate during splicing. This notion is contrary to the spliceosome-retention view articulated above and is based on the observation that some genes, β-globin, for example, are poorly expressed as cDNAs (Buchman and Berg 1988). Also relevant is the fact that some naturally intronless transcripts, like those from the HSV (herpes simplex virus) thymidine kinase gene, contain an RNA element necessary for transport (Liu and Mertz 1995). Similarly, the intronless mouse histone H2a transcript contains a small element that facilitates cytoplasmic accumulation (Huang and Carmichael 1997).

Role of hnRNP proteins in mRNA export

In thinking about nuclear RNP, the current prevailing notion is that many associated RNA binding proteins, so-called hnRNP proteins, facilitate and are perhaps essential for the export of the RNAs to which they are attached (for review, see Nakielny and Dreyfuss 1997; Nakielny et al. 1997). This connection between hnRNP proteins and export is due predominantly to the pioneering experiments of Dreyfuss and colleagues. They showed that many of these proteins are not limited to the nucleus as had been previously believed, but shuttle continuously between nucleus and cytoplasm. Export does not require RNA synthesis, suggesting that the active process is hnRNP or protein export and that the RNA transcripts are passive cargo—analogous to Rev-mediated export (Piñol-Roma and Dreyfuss 1992). Subsequent work has defined a specific export signal on hnRNP A1, called M9, which is distinct from Rev-like NESs (Michael et al. 1995). Therefore, at least some family members are like Rev and have at least two essential domains: an RNA binding region and an effector region that somehow connects to nuclear export. There is even evidence that different shuttling hnRNP proteins have different export signals, which use nonidentical export pathways (Michael et al. 1997). As different primary transcripts are decorated by different sets of hnRNP proteins (Matunis et al. 1993), these may define the nonidentical export pathways used by different mRNAs in the oocyte system (Izaurralde et al. 1997a,b; Pasquinelli et al. 1997a; Saavedra et al. 1997a). The critical role of RNP proteins in RNA export may be equally applicable to ribosome export, in which a specific RNA-binding protein could be the direct export substrate with the RNA coming along for the ride. But it is important to note that, with the exception of the above discussion about Crm1p, no mRNA-relevant export receptors are known. The M9 sequence in hnRNP A1 signals both import and export (Michael et al. 1995), and hnRNP A1 is imported through an interaction with the import receptor transportin (Pollard et al. 1996). Although an import receptor could serve as an export receptor, transportin is unlikely to be involved in hnRNP A1 export (Izaurralde et al. 1997a; Siomi et al. 1997). The relevant export receptor remains unknown (Fig. 2).

Analogous to Rev and splicing, the hnRNP literature also suggests that there is a struggle between RNA-binding proteins that are retained and RNA-binding proteins that are export substrates (for review, see Nakielny et al. 1997). Therefore, nuclear mRNPs might be more difficult export substrates than snRNAs, because some of the bound proteins might promote retention rather than export. In contrast to hnRNP A1, hnRNP C is not exported and does not shuttle. This is probably not just attributable to the absence of an export signal, because a chimeric protein between hnRNP C and the transport competent region of hnRNP A1 fails to shuttle, that is, hnRNP C wins (Nakielny and Dreyfuss 1996; Nakielny et al. 1997). This indicates that some hnRNP retention is active and suggests that bound hnRNP C may impede export of an RNA, or that hnRNP C removal may be a prerequisite for cytoplasmic transport. Consistent with the latter possibility, microscopy experiments in the

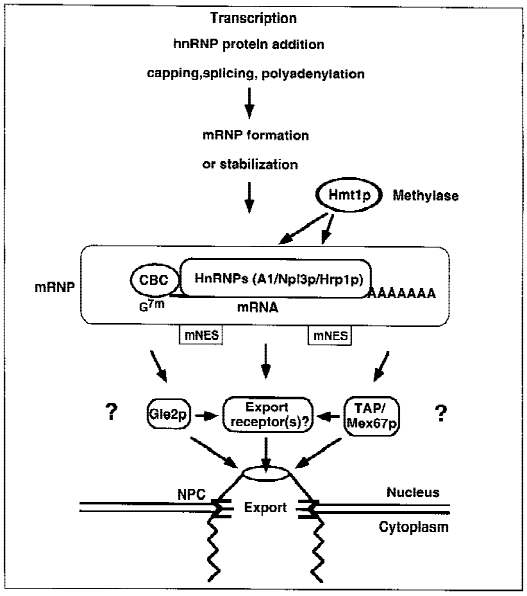

Figure 2. Following transcription, the pre-mRNA is processed and packaged into a mature RNP competent for nuclear export. In yeast, the methylase Hmt1p may participate in the formation and stabilization of the mRNP or the release of the mRNP from the nucleus. With the exception of the hnRNP A1 M9 sequence, the mRNP nuclear export signal(s) (mNES) have not been identified and it is not clear whether an importin-β like receptor is involved in mRNA export. Factors such as Gle2p and TAP1/Mex67p could mediate mRNA export by bridging the mRNP-export receptor interaction or more directly the mRNP-nuclear pore complex interaction. (NPC) Nuclear pore complex (see text for references).

Chironomous balbiani ring system indicate that the insect version of hnRNP C is stripped from the RNP at or near the pore just prior to export. In contrast, the insect version of hnRNP A1 remains part of the RNP during export through the pore (Alzhanova-Ericsson et al. 1996; Visa et al. 1996a). The importance of hnRNP C removal from the RNP transcript at the pore might be analogous to the more speculative notions about spliceosome disassembly articulated above. Moreover, there are enzymatic activities at or near the pore that might contribute to RNP remodeling as well as to RNP export (Snay-Hodge et al. 1998; Tseng et al. 1998).

hnRNP proteins and mRNA export in yeast

All of these considerations reinforce the notion that the nuclear mRNA export substrate is an RNP complex rather than RNA. From a yeast genetic point of view, an RNP network and its relationship to export has been best explored by Silver and colleagues (Henry et al. 1996; Kessler et al. 1997; Shen et al. 1998). This story begins with the yeast hnRNP protein Npl3p. The protein was originally implicated in a number of processes, including RNA export, protein import, and rRNA processing (Bossie et al. 1992; Russell and Tollervey 1992; Singleton et al. 1995). It is predominantly a nuclear protein, resembles by sequence the vertebrate RNA-binding protein hnRNP A1 and has been shown to cross-link to poly(A)$^+$ RNA (Flach et al. 1994; Wilson et al. 1994). It is therefore reasonable to consider it a yeast hnRNP protein. But its RNA-binding RRM domain contains a signature motif typical of the RNA recognition motif (RRM) region of the SR (serine–arginine-rich) family of splicing cofactors (Birney et al. 1993); however, Npl3p presents no obvious SR domain. As there are no bona fide yeast SR proteins, Npl3p might be a bifunctional protein with the functions of the hnRNP A1 family as well the SR splicing family.

Because Npl3p shuttles between the nucleus and cytoplasm and the export of poly(A)$^+$ RNA is blocked in a temperature-sensitive mutant of *NPL3* (Lee et al. 1996), it has been proposed that Npl3p is an RNA export factor, analogous to Rev or to hnRNP A1. But unlike these two proteins, no Npl3p effector domain, relevant to export and separate from the RNA-binding regions, has been identified. Moreover, and also unlike hnRNP A1, Npl3p export is dependent upon ongoing RNA polymerase II transcription (Flach et al. 1994; Lee et al. 1996). This is similar to SR protein shuttling, which requires RNA binding. Moreover, the SR domain does not confer export function on its own (Caceres et al. 1998). Therefore Npl3p could be passive cargo and the RNA (or some other component of the RNP) more directly relevant to export. In this case, the effect of Npl3p temperature-sensitive mutants on poly(A)$^+$ RNA export would be due to mutant effects on the RNP transport substrate. These could cause poor RNP formation, unstable RNP, or otherwise altered nuclear RNP. The mutant proteins could also affect RNP by causing aberrant protein–protein interactions and poisoning other hnRNP or nuclear protein activities. In this case, the temperature-sensitive mutants are more likely to manifest dominant effects, which have not been examined carefully (P. Silver, pers. comm.).

An effect on RNP assembly is well supported by the elegant genetics that Silver and colleagues have done. Through a combination of approaches, they have explored the genetic space around *NPL3* and have come up with a number of genes, all of which can be interpreted to affect nuclear RNP in interesting ways (Fig. 2). The hnRNP protein Hrp1p was identified in a selection for suppressors of a temperature-sensitive mutant of *NPL3*. This nuclear RNA-binding protein was subsequently assigned a precise role in 3′ end formation (Henry et al. 1996; Kessler et al. 1997). It recalls the earlier experiments of Birnstiel and colleagues that indicated an important role for 3′-end formation in the export of the nonpolyadenylated histone mRNAs: Histone mRNAs were efficiently exported when produced by the normal 3′-end cleavage pathway but not when produced by ribozyme cleavage; these data suggested that factors involved in histone RNA 3′-end formation were required for export (Eckner et al. 1991). It also recalls the fact that the 3′ as well as the 5′ends of RNA/RNP appear to make direct contact with nuclear pores prior to export (Mehlin et al. 1992; Kiseleva et al. 1996; for review, see Daneholt 1997).

The protein Hmt1p was identified in a synthetic lethal screen for *NPL3*-interacting genes. It is the major hnRNP methylase and has been shown to methylate both Npl3p and Hrp1p (Henry and Silver 1996; Shen et al. 1998). *HMT1* is inessential. Although the data do not exclude another more minor methylase, they suggest that hnRNP methylation is largely inessential. The fact that *HMT1* overexpression can suppress the temperature-sensitive growth of certain *NPL3* mutant alleles suggests a relationship between the *NPL3* mutant phenotype and the function of hnRNP methylation. As the direct cause of the temperature-sensitive growth of *NPL3* mutants as well as the function of hnRNP methylation is unknown, these results are somewhat difficult to interpret. But a simple idea is that methylation serves to weaken hnRNP RNA binding. The RGG regions of hnRNP proteins, the principal sites of hnRNP methylation (Liu and Dreyfuss 1995), might bind directly to RNA and too tightly in the absence of methylation. Perhaps nuclear RNA binding proteins, even the ones that transit the nuclear pore along with the RNA, need to maintain some conformational flexibility to allow optimal RNA export; the absence of methylation may reduce the efficiency of such rearrangements. If the temperature-sensitive mutant protein fails to adopt a proper conformation and inappropriately acts as a retention signal, this can perhaps be overcome by further weakening the protein's RNA binding with excess Hmt1p activity. According to this scenario, the mutant protein can poison the transport of an mRNA to which it is attached, despite the fact that the wild-type protein has no active or direct role in mRNA export.

An additional genetic step was taken when a synthetic

lethal screen starting with a deletion of *HMT1* came up with the large subunit of the CBC, *CBP80* (Fig. 2). Cbp80p is also inessential, and the double deletion of *CBP80* and *HMT1* is lethal as expected (Shen et al. 1998). Presumably, some feature of nuclear RNP, assembly or stability perhaps, is impacted by the double absence of CBC and hnRNP methylation. Npl3p shuttling is decreased in the absence of *HMT1*, but there is no effect of the deletion on poly(A)$^+$ RNA localization (Shen et al. 1998), suggesting that Npl3p export can be dissociated from bulk mRNA export. Unlike Npl3p, however, Cbp80p continues to shuttle in the absence of *HMT1*. As vertebrate experiments have linked CBC to CRM1, it is possible that this link also exists in yeast and is relevant to yeast messenger RNP as well as snRNA export. But at the moment one can only speculate about links from nuclear RNP to export receptors. Candidates include CBC, Npl3p, Hrp1p and Crm1p, but they remain candidates.

Nuclear pore complex structure and function

To understand fully nucleocytoplasmic transport, it is important to identify the numerous components of the nuclear pore complex (NPC) and to assign them a function. The biochemical and genetic dissection of this macromolecular complex has led to the isolation of a substantial fraction of its components, also called nucleoporins or NUPs (Fabre and Hurt 1997). The overall structure/organization of the NPC is evolutionary conserved and the recent identification of many of its constituents reveals a reasonable conservation between yeast and higher eukaryotes, consistent with the homology observed among the soluble components of the transport machinery. Biochemical purification of nucleoporins has been the main approach in *Xenopus* or mammalian systems; in yeast, biochemistry has been complemented effectively by genetic approaches: experimental genetics as well as completion of the yeast genome project. As a consequence, of the 50 proteins identified in highly purified yeast pore preparations (Rout and Blobel 1993), >30 have now been cloned. Investigations into their function continues. Interestingly, many of these pore proteins are inessential for growth suggesting functional overlaps between various pore components; alternatively, inessential factors may be required to optimize or specialize pore function (for review, see Pante and Aebi 1996a; Doye and Hurt 1997; Fabre and Hurt 1997).

Among the genetic approaches used in yeast to identify new pore components, the synthetic lethal approach has proven to be particularly rewarding (Doye and Hurt 1995). Such screens take advantage of the fact that a conditional mutant in a pore component is viable on its own, but is lethal in combination with a second mutation, presumably in a functionally related gene. Two components may be synthetically lethal because they are in the same complex, perform similar functions, or because they participate in the same pathway.

Initial screens of this type were carried out with mutants of the two first cloned pore genes, *NSP1* and *NUP1* (Wimmer et al. 1992; Belanger et al. 1994), which then led to the identification of a whole series of new pore complex proteins. These then served as starting points for subsequent synthetic lethal screens and identified a complex network of genetic interactions among numerous NPC components. Some of these genetic interactions were subsequently shown to reflect physical interactions. For example, *NSP1* mutants undergo synthetic lethal interactions with *NUP49*, *NIC96*, and *NUP57* mutants (among other nuclear pore genes), and Nsp1p, Nup49p, Nic96p, and Nup57p have been shown to be part of a nuclear pore subcomplex (Grandi et al. 1993). Based on partial sequence conservation, a homologous complex was described in higher eucaryotes and consists of mammalian p62, Nup54, Nup58, and Nup93 (Guan et al. 1995). Another yeast subcomplex was identified by affinity purification with protein A-tagged Nup84p and consists of Nup85p, Nup120p, Sec13p, and Seh1p in addition to Nup84p (Siniossoglou et al. 1996).

Synthetic lethal screens have more recently been expanded to the NPC genes *NUP49*, *NUP100*, *RIP1*, and *NUP85*, all of which identified new as well as already known pore or pore-associated proteins, suggesting that the identification of nucleoporins in yeast is coming close to an end (for review, see Doye and Hurt 1997; Fabre and Hurt 1997). The fact that most of these screens identified proteins restricted to the NPC underscores the power of this genetic approach to identify components of a macromolecular complex. The same observation has been made for spliceosome components (Stutz et al. 1998), and appears to be true for nuclear mRNP (see above comments on RNP).

A useful complementary approach is the analysis of conditional mutants by in situ hybridization with oligo(dT) probes. This searches for mutants that cause nuclear accumulation of poly(A)$^+$ RNA under restrictive conditions. A number of these RAT (mRNA trafficking) or MTR (mRNA transport) mutants turned out to be pore mutants (Amberg et al. 1992; Kadowaki et al. 1992).

NUPs identified in yeast or in higher eukaryotes can be grouped loosely based on their molecular composition and/or location within the pore. The first class consists of integral membrane proteins (yeast Pom152 and mammalian gp210 and POM121), which are believed to make up the core of the NPC and to anchor the complex into the nuclear membrane. The second class, comprised of 10 members in yeast, consists of the phenylalanine–glycine or FG-repeat-containing NUPs. These pore components are thought to be located at the NPC periphery (mainly based on immunoelectron microscopy studies in higher eukaryotes) and are characterized by domains rich in FG, GLFG, or FXFG repeats. These sequences are not NPC targeting signals and are in most cases dispensable for growth, suggesting functional redundancy among different FG-repeat domains. The in vivo function of these regions is not known, but they have been shown to interact with soluble transport factors in vitro, in *Xenopus* extracts and in vivo (Belanger et al. 1994; Radu et al.

1995a; Rexach and Blobel 1995; Fornerod et al. 1997b; Shah et al. 1998). Many other pore proteins do not contain FG repeats but contain other motifs: These include coiled-coil domains, heptad repeats, zinc fingers, leucine zippers, and some sequences consistent with RNA-binding domains (for review, see Rout and Wente 1994; Davis 1995; Pante and Aebi 1996b; Doye and Hurt 1997; Fabre and Hurt 1997).

Although yeast appears to be a system of choice for identifying NPC components, the next challenge is to assign specific functions to each protein. Clues as to how nucleoporins function in vivo can be obtained from phenotypic analysis of nucleoporin mutants. These analyses include in vivo tests for defects in nuclear protein import, poly(A)$^+$ RNA export and diffusion of proteins through the NPCs (Amberg et al. 1992; Kadowaki et al. 1992; Saavedra et al. 1996; Shulga et al. 1996). Functional analysis, however, is complicated by the fact that the role of many nucleoporins may be primarily structural with only a fraction playing a more specific role in transport. Another problem comes from the fact that nuclear pores serve as the transit site for macromolecules both entering and exiting the nucleus. As described above, effects on import may also indirectly affect export. Despite these complications, a subset of nucleoporins has been assigned a primary role in RNA export. This is based largely on the fact that conditional mutants induce a rapid block in poly(A)$^+$ RNA export without a concommitant block in import. Such genes have been identified in the temperature-sensitive mutant screens for a specific block in poly(A)$^+$ RNA export as well as in other more traditional genetic screens. They comprise *NUP84*, *NUP85/RAT9*, *RAT2/NUP120*, *NUP159/RAT7*, and *RAT3/NUP133*. Note that these include three members of a nuclear pore subcomplex mentioned above. It will be interesting to determine the location of this subcomplex within the pore, if it is associated with the nuclear baskets for example. It is also of interest to define whether some of these pore components are present at more than one location, indicative perhaps of a more dynamic role during transport.

NPC-associated components with a role in RNA export

Several other pore or pore-associated proteins have more recently been proposed to have primary roles in RNA export. One is the nonessential protein Gle2p/Nup40p that directly associates with the FG-nucleoporin Nup116p. *GLE2* mutants are synthetic lethal with a *NUP100* disruption (Murphy et al. 1996; Bailer et al. 1998). Gle2p and its *S. pombe* homolog, Rae1p, are located at the pore with some fraction in the cytoplasm, and mutations in these factors induce a rapid block in mRNA export (Brown et al. 1995; Murphy et al. 1996; Bailer et al. 1998). The function of Gle2p is coupled to its interaction with a short region from Nup116p that targets Gle2p to the pore. In the absence of this interaction, cells exhibit NPC herniations with a membrane seal over the pores typical of both *NUP116* and *GLE2* mutants; it is thought that these morpholigical perturbations are secondary to an RNA export defect because their appearance follows the nuclear accumulation of poly(A)$^+$ RNA (Wente and Blobel 1993; Murphy et al. 1996; Bailer et al. 1998). The Nup116p sequence targeted by Gle2p is conserved in vertebrate nuclear pore proteins related to Nup116p, such as Nup98, indicating that the docking of the Gle2p/Rae1p protein family to nuclear pores might occur through a conserved mechanism (Bailer et al. 1998). Although a physical interaction between Gle2p/Rae1p and RNA has not yet been demonstrated, a human homolog of Gle2p, MRNP41, was identified by cross-linking to poly(A)$^+$ RNA. It is localized at the pore and in the cytoplasm, possibly in association with cytoskeletal elements. It was suggested that MRNP41 might participate in the cytoplasmic transport of mRNPs along cytoskeletal elements as well as in some feature of mRNP export (Kraemer and Blobel 1997).

The synthetic lethal screen with a null allele of *NUP100* also identified Gle1p as a candidate RNA export mediator (Murphy and Wente 1996). Gle1p was cloned independently as a high-copy suppressor of a *RAT7/NUP159* temperature-sensitive mutant (Del Priore et al. 1996), in synthetic lethal screens with Rip1p (Stutz et al. 1997) and the Nup1p (M. Kenna and L. Davis, unpubl.) pore proteins, as well as in a screen for cold-sensitive mutants exhibiting splicing and poly(A)$^+$ RNA export defects (Noble and Guthrie 1996). Consistent with these genetic interactions, Gle1p localizes mainly to the NPC (Del Priore et al. 1996; Murphy and Wente 1996). Intriguingly, Gle1p was shown to contain an essential Rev-like NES required for mRNA export (Murphy and Wente 1996). However, Gle1p has not been shown to bind RNA nor to shuttle, and no connection has yet been established between Gle1p and the NES receptor Crm1p. This raises the question of whether the Gle1p NES acts as a nuclear export signal in the context of the whole protein or whether this sequence plays another role in yeast mRNA export. Interestingly, a human homolog of Gle1p, involved in mRNA export in mammalian cells, contains no NES and is able to complement a yeast *GLE1* temperature-sensitive mutant. Function in yeast however requires that a Rev-like NES be inserted into the region of the human protein that aligns with the NES from yGle1p (Watkins et al. 1998). This result underscores the functional importance of the yeast Rev-like NES region, but it does not clarify whether this NES-like sequence is really a nuclear export signal or whether it is important for another aspect of Gle1p function in yeast, such as localization through an association with other pore proteins.

Another factor with a direct role in mRNA export, called Mex67p, was identified in a synthetic lethal screen with an amino-terminal deletion mutant of *NUP85*, which exhibits an RNA export defect (Segref et al. 1997). Mex67p mainly localizes at the pore and probably plays a direct role in mRNA export because temperature-sensitive alleles of *MEX67* show a very rapid block in poly(A)$^+$ RNA export with no apparent import block. Importantly, Mex67p cross-links to poly(A)$^+$ RNA

despite the absence of a previously identified RNA-binding domain. It is not clear whether Mex67p shuttles between nucleus and cytoplasm to perform its transport function or whether it remains at the pore. As mutant forms of Mex67p mislocalize to either the nucleus or cytoplasm, the association of Mex67p with the pore may be transient and dynamic. Mex67p also contains an essential carboxy-terminal Rev-like NES, and mutations therein interfere with the association of Mex67p and the nuclear pore and interfere with poly(A)$^+$ RNA export. However, it is not clear whether this sequence acts as a bona fide NES or whether its homology to Rev-like NESs is fortuitous (Segref et al. 1997). In this context, TAP, the human homolog of Mex67p, contains no Rev-like NES (Grüter et al. 1998; Segref et al. 1997).

Human TAP was recently and independently identified as the cellular factor recruited by the CTE (constitutive transport element) of type D retroviruses (Grüter et al. 1998). These simple retroviruses do not encode a Rev-like *trans*-acting protein but still must export unspliced and poorly spliced transcripts like complex retroviruses. This process relies on the interaction of a *cis*-acting RNA element (the CTE) with cellular factors (Bray et al. 1994; Tabernero et al. 1997). TAP was shown to directly bind to the CTE and to promote the export of CTE-containing transcripts in the *Xenopus* oocyte system (Grüter et al. 1998). Interestingly, injection of an excess of CTE RNA blocks mRNA export, indicating that TAP is also an essential mediator of cellular mRNA export (Pasquinelli et al. 1997a; Saavedra et al. 1997a). Noteworthy is the observation that an excess of hnRNP A1, which saturates the export of specific mRNAs, does not interfere with CTE-mediated export. This suggests that TAP promotes RNA export at a step following the interaction of certain mRNAs with hnRNP A1 (Izaurralde et al. 1997a; Saavedra et al. 1997a; Grüter et al. 1998). These data also indicate that the binding of TAP to the CTE is able to bypass certain steps in the mRNA export pathway and to override nuclear retention mechanisms. TAP has been purified from nuclear extracts, indicating that at least a fraction of this protein is soluble and supporting a transient association with the pore. It will be interesting to determine whether TAP interacts directly with messenger RNAs and whether it might have a regulatory role by targeting specific mRNA sequences for export.

A final interesting player in poly(A)$^+$ RNA export is the recently described *DBP5/RAT8* gene, which encodes a member of the DEAD box family of RNA helicases. A temperature-sensitive allele of *RAT8* was identified initially in the screen for poly(A)$^+$ RNA retention mutants (RAT mutants) and concomitantly because of its similarity to other RNA helicases (Snay-Hodge et al. 1998; Tseng et al. 1998). *RAT8* mutants show a much faster and stronger nuclear accumulation of poly(A)$^+$ RNA than the previously described helicase Mtr4p, which has been suggested to play a role in mRNA export (Liang et al. 1996; Snay-Hodge et al. 1998). Dbp5p/Rat8p is found in the cytoplasm, but a substantial fraction is associated with NPCs. The link between Dbp5p/Rat8p and RNA export is strengthened by the absence of an import defect and by the identification of synthetic lethal interactions with the nucleoporins Rat7p/Nup159p, Rat9p/Nup85p, Rat10p/Nup145p, and Rip1p/Nup42p, which have been independently implicated in export. There are also genetic interactions with the export/transport factors Gle1p/Rss1p and Rna1p (Snay-Hodge et al. 1998). Dbp5p/Rat8p has RNA-dependent ATPase activity and RNA helicase activity in vitro, but the latter requires an additional factor (Tseng et al. 1998). The identification of this RNA-dependent ATPase is interesting because very few enzymes involved in RNA export are known. The presence of several potential NESs within Dbp5p/Rat8p raises the possibility that it shuttles and might accompany RNPs during the export process together with the targetting components. Its enzymatic activity might serve to induce required RNP rearrangements at the pore before export, or in the cytoplasm to allow recycling of shuttling RNA-binding proteins. All these unfolding or rearrangement processes may be energy dependent. The export of several substrates, including tRNA, NES-proteins, and importin-α, have been shown to be dependent on Ran–GTP, but GTP hydrolysis was not required for export. These observations suggest that a different energy source, perhaps ATP hydrolysis, may be used for the export of certain substrates (Izaurralde et al. 1997b; Richards et al. 1997).

Heat shock RNA export

Heat or ethanol stress blocks normal poly(A)$^+$ RNA export, but heat shock (HS) RNAs are exported efficiently under these conditions. This process is apparently independent of the Ran regenerating system (Saavedra et al. 1996): Mutations in the Ran–GAP Rna1p and in the nucleotide exchange factor RCC1/Prp20p, as well as high level expression of a dominant-negative Ran (Gsp1p) mutant locked in its GTP-bound form, do not interfere with HS RNA export. One possibility is that another GTPase is involved in HS shock RNA export. Alternatively, HS RNA export may only require low levels of nuclear Ran–GTP, in which case the low residual amounts of nuclear Ran–GTP in the *prp20-1* mutant would be sufficient. Also, the apparent absence of a requirement for the Ran–GAP (insensitivity to the *rna1-1* mutant) suggests that release of HS RNPs in the cytoplasm may occur through a different process or involve other components (for review, see Cole and Hammell 1998). The HS RNA export pathway is also independent of Npl3p activity (Saavedra et al. 1997b).

In contrast, this pathway is sensitive to mutations in several other factors also important for non-HS RNA export, such as the NUP genes *RAT7/NUP159*, *RAT9/NUP85*, *RAT2/NUP120*, or *RAT10/NUP145*, and the transport gene *GLE1/RSS1*. Interestingly, HS RNA export is competed by high-level expression of wild-type Rev but not by an export-defective Rev mutant, suggesting that HS RNP export may depend on an NES (Saavedra et al. 1997b). Consistent with this possibility, HS

RNA export is reduced in a temperature-sensitive *CRM1* mutant background (F. Stutz, unpubl.). Finally, HS RNP export under stress conditions absolutely requires the otherwise inessential FG–nucleoporin Rip1p. In contrast, *Gal1* promoter-driven HS RNAs expressed at 25°C or HS RNAs induced at 37°C are exported through a pathway independent of Rip1p (Saavedra et al. 1997b; Stutz et al. 1997), indicating that there is something special about the stress conditions rather than the transcript. The FG-repeat region of Rip1p, initially identified as a Rev NES interacting region in a two-hybrid screen, was proposed more recently to interact with Rev NES via the bridging activity of the export factor Crm1p (Stutz et al. 1995; Neville et al. 1997; see above). Taken together, these data implied a dedicated role for the FG-repeat region of Rip1p under stress, perhaps as a docking site for HS RNPs. It turned out, however, that the unique carboxy-terminus of Rip1p rather than its FG-repeat domain is essential for HS RNA export. The carboxyl terminal region of Rip1p is also able to rescue the synthetic lethality of *GLE1* mutations and a *RIP1* disruption, pointing to a parallel role of this domain in non-HS RNA export (Stutz et al. 1997). Although the function of this region is not yet understood, both genetic and two-hybrid data indicate that it physically interacts with Gle1p (F. Stutz, unpubl.). We cannot exclude the possibility that Rip1p may be involved in the reimport of factors essential for HS RNA export. As NLS-dependent import is apparently blocked under heat shock, these factors may be imported via one of the more recently identified NLS-independent pathways. This possibility also applies to Gle1p. However, we favor the notion that Rip1p as well as Gle1p optimize pore function for RNA export, especially for heat shock RNA export.

Concluding remarks

Numerous transport components have been identified, and many of them have been related to specific export or import phenotypes. With the exception of Ran and a few export and import receptors, however, the biochemical function of most of these components remains a mystery. The precise localization of a protein within the NPC, its assignment to specific subcomplexes, and its possible movements within or between pore substructures may bring additional clues to a precise role in transport. In the case of export, progress also awaits an in vitro system that can be exploited for biochemical complementation.

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