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Nuclear Export Receptors: From Importin to Exportin

Minireview

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What mediates export from the nucleus? What recognizes the diverse array of nuclear export cargoes, from mRNA to the shuttling proteins IκB and Rev? One would predict that nuclear export receptors exist to fill this role, but data to support this prediction has been a long time coming. Before describing the suddenly rich selection of export receptors described in this issue of Cell and elsewhere, a brief review of the preceding knowledge is needed to set the stage.

Nuclear Import

The rules and players of nuclear import are now becoming known. Nuclear import involves: NLS recognition, pore docking, translocation through the pore, and release from the inner side of the pore (Powers and Forbes, 1994; Görlich and Mattaj, 1996; Doye and Hurt, 1997). Typically, nuclear proteins possess a short positively charged nuclear localization sequence or NLS, such as that of the SV40 T Ag, which ensures their import. The NLS receptor has been identified and consists of the soluble proteins importin α and importin β, which form a heterodimer (Table 1). Importin α recognizes the NLS of a future nuclear protein, while importin β mediates interaction with the nuclear pore.

The small GTPase, Ran, is required for nuclear import (Goldfarb, 1997). Strong in vitro evidence indicates that RanGTP dissociates importin α from importin β (Figure 1, top panel). In vivo, RanGTP may be used to promote translocation of the NLS-protein/α/β complex across the pore, to release the complex from the nuclear side of the pore, or both. Strikingly, the roles of importin α, β, and Ran in import have been conserved from yeast to humans (Doye and Hurt, 1997).

Interestingly, there are other pathways for nuclear import that are guided by noncanonical nuclear localization signals. The hnRNP A1 protein, which shuttles continuously between nucleus and cytoplasm, contains a very different sequence, termed M9, which allows its import. The M9 localization sequence binds to a newly identified import receptor, transportin, related in sequence to importin β (Pollard et al., 1996). Significant functional divergence has occurred, however: transportin in a single protein fulfills both the signal recognition role of importin α and the pore docking role of importin β.

In yeast, a transportin equivalent has been discovered through a search of the S. cerevisiae genome (Aitchison et al., 1996). Yeast transportin binds in vitro to two mRNA binding proteins and is essential for their import (Table 1). The search for importin β–related proteins revealed two additional relatives, Kap123p and Pse1p (Rout et al., 1997). A knockout of *KAP123* is deficient in ribosomal protein import and can be suppressed by overexpression of *PSE1*, implicating these proteins as potential ribosomal import receptors.

The important conclusion is that the import receptors for a number of the major nuclear localization pathways are becoming known (Figure 2, top). It is the clear expectation that additional receptors for other import pathways will also be found.

Nuclear Export

RNAs exit the nucleus through the nuclear pore. Elegant electron microscopy by the Feldherr, Daneholt, and Allen groups has allowed direct visualization of RNA export (see Daneholt, 1997). In both the nucleus and the cytoplasm, RNAs are complexed with protein and it is thought that the protein components, perhaps specific for each class of RNA, could well provide the signal for export. Indeed, it was found that the export of each RNA class (mRNA, 5S rRNA, tRNA, snRNA, and rRNA) is uniquely saturable (reviewed by Görlich and Mattaj, 1996).

One of the earliest compelling indications that proteins mediate RNA export came from HIV-1. Unspliced mRNAs encode important viral proteins at certain stages of the life cycle. How does this unspliced RNA exit the nucleus when it would normally be retained? The protein responsible is the ~13 kDa viral Rev protein, which recognizes a specific HIV RNA sequence, the RRE, and mediates export of the unspliced viral RNA. Fischer et al. and Wen et al. (reviewed by Gerace, 1995) identified a "nuclear export signal" or "NES" within the Rev protein, the leucine-rich sequence, LPPLERLTL. Excitingly, excess NES conjugate also blocked the export of 5S rRNA and U1 snRNA, leading to the conclusion that an NES is present on proteins bound to 5S rRNA and U1 snRNA and signals their export. A second conclusion was that the viral Rev protein must access this normal cellular RNA export pathway when it shepherds HIV-1 unspliced RNA out of the nucleus. Interestingly, the Rev NES sequence used by Fischer and colleagues failed to inhibit the export of mRNA or tRNA, leaving the mechanism of export for these RNAs a mystery, one that remains to date.

Work by the Hope and Cullen laboratories has carefully delineated the critical features of an NES (reviewed by Hope, 1997). Proteins ranging from I-κB to MAPKK contain potential NES peptides, and it is becoming increasingly clear that control of their import and export is important for regulating their function.

The Search for an Export Receptor

The search began in earnest for an export receptor. One tact was to use a yeast two-hybrid system to search for proteins that interact with Rev. In this way, the Green, Rosbash, and Cullen laboratories identified related proteins in yeast and human cells, named variously Rip or Rab (see Gerace, 1995, for review). An involvement of Rip/Rab in Rev export was made more convincing by their possession of the "FG repeats" characteristic of a subclass of nucleoporins. However, a critical criterion for an export receptor, i.e., direct interaction with the NES, remains to be fulfilled.

Several years ago, yeast genetic screens were set up by the Tartakoff and Cole laboratories to identify proteins involved in mRNA export (see references in Doye

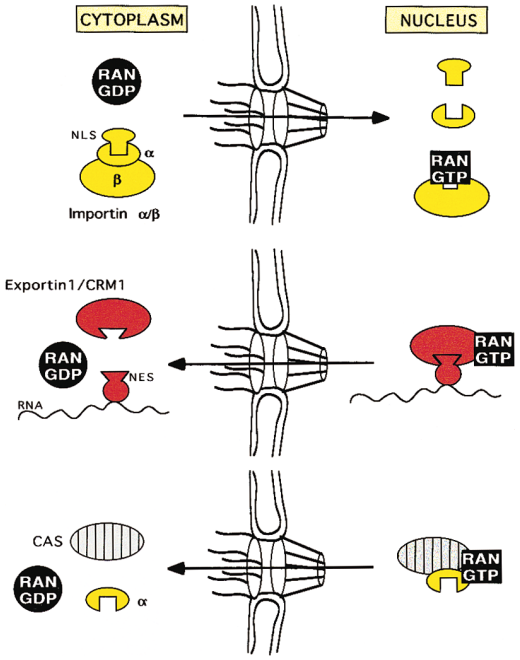

Figure 1. Ran-GTP in Import and Export

RanGTP may ensure that export complexes assemble in the nucleus, while import complexes disassemble there. RanGTP is predicted to be at high concentration in the nucleus.

and Hurt, 1997). Looking for mutations that cause temperature-sensitive accumulation of poly(A)$^+$ mRNA, this screening method has successfully identified mutations in known transport-related factors, such as RCC1, and in multiple nucleoporins, but did not immediately yield a recognizable export receptor.

Seedorf and Silver (1997) have now used the poly(A)$^+$ accumulation assay to focus on whether importin β–related proteins might participate in nuclear export. Mutants of importin β itself and of transportin have little effect on yeast RNA export. Thus, it was surprising and exciting for Seedorf and Silver (1997) to find that conditional loss of the importin β–related Pse1p in a strain deleted for *KAP123* resulted in a very rapid block in RNA export. These authors could find no defect in protein import, although they did not analyze the ribosomal proteins observed to be affected by Rout et al. (1997; Table 1). Their overall conclusion is that the importin β–related proteins, Pse1p and Kap123p, are both used for RNA export. This redundancy of export function was brought home even more strongly by the finding that overexpression of a new importin β relative, *SXM1*, partially suppresses the RNA export defect (Figure 2, Table 1). Thus, members of the importin β family are involved not only in protein import, but also in RNA export.

Identification of an even larger importin β superfamily, based on sequence homology with the N-terminal 150 residues of importin β, now gives one a potentially rich source to find more export and import receptors. The β family includes ~20 proteins from yeast to humans (Fornerod et al., 1997a; Görlich et al., 1997). The challenge is to determine whether they are transport receptors and, if so, to identify their cargoes. For two, CRM1 and CAS, the challenge has been met in this issue of Cell.

CRM1: A Long-Awaited Export Receptor

Clues pointing the way to CRM1 previously existed. Fornerod et al. (1997a) found that human CRM1 bound to the nucleoporin CAN/Nup214 and other proteins within the pore. The association of CRM1 with the pore was dynamic, suggesting that CRM1 shuttled, but no firm data had yet been found that it was an export receptor.

A second clue lay in a recent report which divulged that an experimental drug, leptomycin B (LMB), could block Rev export and Rev-mediated RNA export in tissue culture cells (Wolff et al., 1997). A previous study on leptomycin B–resistant mutants of S. pombe revealed that one class of mutants fell in the essential gene, *CRM1*. The involvement of *CRM1* was confusing with respect to export, as *CRM1* was originally identified as a gene required for *c*hromosome *r*egion *m*aintainence

Table 1. Importins and Exportins

Importins		Other Names
α family		
Importin α	Binds NLS-bearing proteins = NLS receptor	Karyopherin α Kap60, Srp1
β family		
Importin β	Partner of importin α	Karyopherin β Kap95, Kapβ1
Transportin	Import receptor for hnRNP A1	Kap104, Kapβ2, MIP
Kap123	Import receptor for ribosomal proteins	Kap β3
Pse1	Suppresses import defect of Kap123 null	Kap121
Exportins		**Other Names**
β family		
Exportin 1	Exports HIV Rev and snRNAs	Crm1, Xpo1
CAS1/Cse1	Exports and recycles importin α	
Pse1 Kap123	Yeast double mutants fail to export mRNA	Kap121
Sxm1	Suppresses mutant Pse1/Kap123 mRNA export defect	
Mtr10	Predicted exportin Mutant fails to export mRNA	

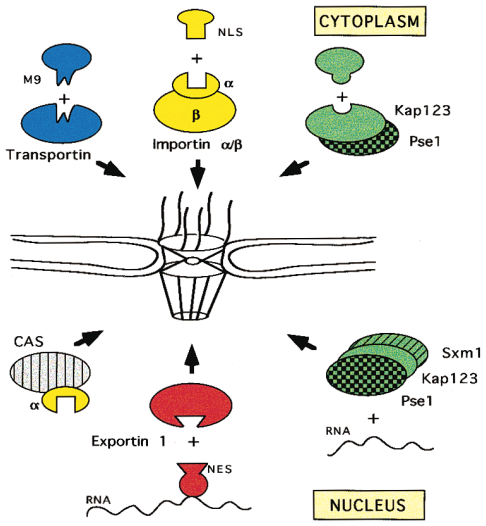

Figure 2. Import and Export Receptors

Import receptors are shown at the top, while export receptors are shown below (see also Table 1).

(see Fornerod et al., 1997b [this issue of *Cell*], and references therein), and also caused defects in regulation of the yeast transcription factor AP1.

Using these clues, Fornerod et al. (1997b) set out to test whether CRM1 could be the export receptor. They found that leptomycin B blocks Rev export from Xenopus oocyte nuclei and that it also blocks export of at least one class of RNA, the U snRNAs. Gel shift analysis showed that leptomycin B interacted directly with CRM1. Furthermore, overexpression of human CRM1 counteracted the effect of the LMB and increased the export of Rev protein and U snRNAs in drug-free oocytes. Most importantly, the authors found that a NES/CRM1/Ran complex forms in the presence of RanGTP. A last piece of the puzzle fell into place when they found that LMB blocks formation of this complex. These findings provide strong evidence that CRM1 is a nuclear export receptor that recognizes Rev-like NESs. The protein has been renamed, accordingly, exportin 1.

The cytoplasmic localization of the RanGAP and the intranuclear location of Ran's GDP-GTP exchange factor RCC1 have led to the hypothesis that there is a gradient of RanGTP across the nuclear envelope, with RanGDP in the cytoplasm and RanGTP in the nucleus (Richards et al., 1997; reviewed by Goldfarb, 1997). Such a distribution is consistent with RanGTP promoting the formation of the CRM1/NES export complex, while breaking apart import complexes (Figure 1).

Pleasingly, experiments in S. cerevisiae led to the identical conclusion that CRM1 is an exportin (Stade et al., 1997 [this issue of *Cell*]). These authors, focusing on *CRM1* because of its relatedness to importin β, showed that CRM1-GFP protein shuttles between nucleus and cytoplasm. They then constructed an elegant import/export substrate, NES-GFP-NLS. In wild-type yeast, this substrate shuttles continuously between the nucleus and cytoplasm, but appears largely cytoplasmic, presumably due to a higher rate of export. However, in yeast containing a ts mutation in *CRM1* (i.e., *xpo1–1*), the substrate became nuclear within 5 min at high temperature. Thus, the *crm*^{ts} mutation blocks NES export. Importantly, the authors found that mRNA export was also blocked at high temperature. Two-hybrid analysis showed an interaction between the NES and CRM1 and, moreover, between Ran and CRM1.

One is clearly left with the conclusion from both the Xenopus and yeast results that CRM1/exportin 1 is an export receptor. It interacts directly with both NES sequences and Ran (Figure 1). The previous pleiotropic phenotypes of *crm1* mutants: defects in chromosome maintenance and effects on AP1 activity, could be the result of an inability to export crucial proteins, leading to abnormal nuclear structure and function. It is striking that, in Xenopus, LMB does not inhibit mRNA export, while in yeast, an exportin 1 mutation does block its export. Perhaps exportin 1 has to handle more cargoes in yeast than in vertebrates, which may have evolved a separate exportin for mRNA. Alternately, the leptomycin-induced defect may not be as severe as that caused by the *xpo1–1* mutation. Both systems should provide fertile ground for testing the mechanism of exportin 1 action and the extent of its interaction with different cargoes.

CAS: An Exportin for Importin α

An additional type of cargo is routinely exported from the nucleus: the import receptors themselves that recycle to the cytoplasm after each round of import. Reasoning that a recycling protein for importin α would bind it in the presence of RanGTP, Kutay et al. (1997 [this issue of *Cell*]) passed cytosol over a column of RanGTP. They found that importin α did indeed bind to RanGTP but that its binding required another protein. Unexpectedly this was revealed to be the cellular apoptosis susceptibility or CAS protein, again a member of the importin β superfamily. Recombinant CAS, Ran-GTP, and importin α were produced and shown to interact only as a heterotrimeric complex. Moreover, CAS protein bound preferentially to NLS-free importin α, as would be predicted of a recycling receptor. The CAS/Ran-GTP/importin α complex could be disassembled in vitro by RanGAP and its cofactor RanBP1, proteins found in the cytoplasm and on the cytoplasmic filaments of the pore. Lastly, and most importantly, importin α recycling was highly dependent on the presence of CAS.

Human *CAS* is 40% homologous to the essential yeast gene *CSE1*. *CSE1* mutations were first identified as ones that interfere with accurate chromosome segregation and that cause a defect in B-type cyclin degradation, both of which take place in the yeast nucleus. *CAS* was identified as a gene whose partial suppression in human cells protects against tumor necrosis factor–induced apoptosis (see references in Kutay et al., 1997). All of these phenotypes in retrospect may be explained by a depletion of importin α in the cytoplasm and the consequent loss of import function. The firm conclusion is that the CAS/Cse1 protein is an exportin that recycles importin α.

What Bridges RNA to Exportin 1?

Clearly the export receptor, exportin 1, appears to recognize an NES as the signal for export. The next relevant question then becomes what bridges the interaction between RNA and exportin 1? For cellular RNAs, the

cap binding complex, CBP20 and CBP80, has been invoked as potentially containing a NES signal for export of mRNAs and snRNAs. The hnRNP A1 protein, which commonly complexes with precursor and mature mRNAs, has also been found to carry a signal for export and may perform this bridging role (reviewed by Görlich and Mattaj, 1996). Mex67p, Npl3p, Gle1p, Gle2p, and TFIIIA are other candidates for such a role (see Doye and Hurt, 1997). Recently, cellular RNA helicase A was found potentially to fill a bridging role, analogous to that of Rev, for the simian retrovirus MPMV (Tang et al., 1997). Many bridging proteins could yet be identified.

Where Do Export Receptors Go Next?

In a literal sense, export receptors may next interact with proteins of the nuclear pore (for example, Powers et al., 1997; see also Doye and Hurt, 1997). Another option is that other soluble factors may be required to bind exportins to the pore. Rip/Rab may belong to an as-yet-uncharacterized class of proteins that act as "itinerant" nucleoporins, soluble at some times and docked at the pore at others. By forming a Rip/exportin 1/Rev-NES complex, such proteins could act to dock the exportins at the pore. Indeed, evidence for such a complex was recently found (Neville et al., 1997).

In the broader sense of where export receptors go next, questions for the future include: Do more exportins exist? If so, are they importin β–related or different? Are there specialized exportins that have distinct cargoes? Does signal transduction alter the exportins used? With exportins now in hand, meaningful experiments on the mechanism of export will soon follow.

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