Transport pathways of macromolecules between the nucleus and the cytoplasm

Stephen A Adam

Transport between the nucleus and cytoplasm involves both stationary components and mobile factors acting in concert to move macromolecules through the nuclear pore complex. Multiple transport pathways requiring both unique and shared components have been identified. In the past 18 months, new findings have shed light on the nature of some of the mobile components of these pathways. New receptor–cargo pairs for both import and export pathways have been identified extending the breadth of known transport pathways. Surprising findings on the role of Ran and energy in transport have changed our way of thinking about the mechanism of movement through the nuclear pore.

Addresses
Department of Cell and Molecular Biology, W129, 303 East Chicago Avenue, Northwestern University Medical School, Chicago, IL 60611, USA; e-mail: s-adam@nwu.edu

Current Opinion in Cell Biology 1999, **11**:402–406

http://biomednet.com/elecref/0955067401100402

 ISSN 0955-0674

Abbreviations

arm	*armadillo*
GDI	guanine nucleotide dissociation inhibitor
IBB domain	importin β binding domain
MAP kinase	mitogen-activated protein kinase
m_3G	trimethyl guanosine
NES	nuclear export signal
NLS	nuclear localization signal
NTF2	nuclear transport factor 2
RanBP1	Ran binding protein 1
RCC1	regulator of chromosome condensation 1

Introduction

A family of transporter proteins known as importins or exportins (both also called karyopherins) mediates the movement of proteins and RNA between the cytoplasm and nucleus [1–3]. Proteins or RNAs to be transported (cargo) contain nuclear localization signal (NLS) sequences or nuclear export signal (NES) sequences that are recognized by importins or exportins, respectively. The interaction of a cargo with its transporter is modulated by the small GTPase Ran [1,2,4–6]. For importins, the binding of cargo and Ran•GTP is antagonistic and for exportins the binding of cargo and Ran•GTP is co-operative. The nucleotide bound state of Ran, in effect, determines the identity of the compartment (Ran•GTP in the nucleus and Ran•GDP in the cytoplasm) and allows the transporter to bind or release the cargo in the proper compartment. A number of accessory factors in the Ran GTPase cycle — including RCC1 (the nuclear nucleotide exchange factor), RanGAP (the cytoplasmic Ran•GTPase activating protein), RanBP1 and the nuclear transport factor 2 (NTF2) — assist in the transport pathway by maintaining Ran in its proper GTP or GDP bound states or by modulating the interaction of Ran with the transporters. In the yeast *Saccharomyces cerevisiae*, 14 members of the importin/exportin family are predicted from the genomic sequence [3]. Fewer transporters have been identified in higher eukaryotes but it seems likely that many more transporters will be found in coming years as more genomic sequence data become available. The redundancy of transporter use and some intriguing differences between yeast and higher eukaryotes in several pathways poses interesting questions on the evolution of both transporters and the signal sequences they recognize [7,8].

Importins and exportins

The importin/exportin family of proteins shares several features including an amino-terminal Ran-binding domain and the ability to shuttle between the cytoplasm and nucleus. Because of the sequence similarity of these proteins, particularly within the amino-terminal Ran-binding domain, the identification of putative transporters in the sequence databases has preceded their functional characterization. In the past year, cargoes for several previously uncharacterized transporters have been identified. A listing of characterized transporters is shown in Table 1.

Unique among this family of transporters is the exporter for tRNA called exportin-t in higher eukaryotes [9••,10••] and Los1p in yeast [11•,12]. The unique feature of this transporter is its direct binding to the tRNA: the first transporter to recognize an RNA rather than a protein localization signal. *LOS1* had previously been implicated genetically in tRNA processing but a direct demonstration of its function had not been made. Like other exporters, the interaction of exportin-t with its cargo and Ran•GTP is co-operative. Exportin-t alone binds tRNA weakly but in the presence of Ran•GTP the affinity of both exportin-t and Ran•GTP for tRNA increases 100-fold. Consistent with its role in transporting mature tRNA molecules to the cytoplasm, exportin-t binds 10-fold more tightly to mature tRNAs than to bacterially expressed precursor tRNAs. As no other RNA-binding proteins appear to be directly involved in tRNA export, this difference in affinity would ensure that mature tRNAs are preferentially exported to the cytoplasm. Los1p is not essential in yeast, suggesting that other transporters may provide redundancy in the tRNA export pathway [13].

Several other transporter pathways have been delineated in the past year. A previously uncharacterized transporter homolog, the *S. cerevisiae MSN5* gene product, was shown to be an exportin for the yeast transcription factor Pho4p [14•]. The export sequence of Pho4p is regulated by phosphorylation as only phosphorylated Pho4p binds Msn5p. This is the first demonstration that an NES can be regulated by

Table 1

Nuclear transporters.

SGD name	Importin/exportin	Other names	Substrates
KAP95	Importin	Importin β1/Karyopherin β1	Importin α/Karyopherin α – classical NLS pathway, Arginine-NLSs, ribosomal proteins
KAP104	Importin	Transportin, Importin β2/Karyopherin β2	mRNA binding proteins – M9 NLS, Nab2p
PSE1	Importin	RanBP5/KAP121/Karyopherin β3	Ribosomal proteins
KAP123	Importin	YRB4/Karyopherin β4	Ribosomal proteins
SXM1	Importin	KAP108 (RanBP7/RanBP8?)*	Lhp1p, ribosomal proteins
MTR10	Importin	KAP111	Hrp1p, Npl3p
NMD5	Importin	(RanBP7/RanBP8?)*	Hog1p MAP kinase
CSE1	Exportin	CAS/KAP109	Importin α/Karyopherin α – IBB domain
CRM1	Exportin	Exportin 1/Xpo1/Kap124	Leucine-rich NESs
LOS1	Exportin	Exportin-t	tRNA
MSN5	Exportin	?	Pho4p
PDR6	?	?	?
YGL241W	?	?	?
YPL125W	?	?	?

The *Saccharomyces* Genome Database (SGD) designations for the 14 predicted transporter genes are in the left column. The transporters listed under 'Other names' include transporters identified in vertebrates and *S. cerevisiae*. These represent only a partial list of names used to describe the proteins but are the most widely used. *It is not clear from the sequence analysis which of these proteins (if either) is the direct homolog.

phosphorylation of adjacent amino acids as has been demonstrated for NLSs [15]. Ferrigno and coworkers [16] demonstrated that the putative transporter Nmd5p is required for nuclear localization of the phosphorylated Hog1p (high osmolarity glycerol 1) MAP kinase and that the export of the dephosphorylated protein requires exportin 1 (Xpo1p/Crm1p). Although a direct interaction of Hog1p with either transporter has not been demonstrated, none of the other yeast transporter homologs are required for translocation of the protein. In yeast, Cse1p has also been confirmed in functional assays as the yeast homolog of the human importin α exporter CAS [17,18].

The classical nuclear localization signal pathway

The family of importin α proteins functions as adapters to link proteins containing the 'classical' NLSs to importin β1 [2]. Although there is a single importin α in *S. cerevisiae*, higher eukaryotes have multiple members of this family that exhibit both NLS-binding specificity and tissue-specific expression [19–22]. All importin αs are characterized by a large central domain of 8–10 *armadillo (arm)* repeats flanked by relatively short amino and carboxyl termini. An amino-terminal domain, rich in basic amino acid residues, called the importin β binding (IBB) domain acts as an NLS by binding importin α to importin β1 [23,24]. The acidic carboxyl terminus of the protein is required for export of importin α by the exportin CAS [25•].

Although importin α was the first transport factor identified and sequenced, its characterization has lagged behind that of other factors. The mechanism for the recognition of NLSs by importin α has been an enigma because of the diversity in the sequence of NLSs recognized by the importin α family. The structural basis for recognition of at least one type of NLS by one of the importin α family is now known from crystallographic analysis of the *arm* repeat domain bound to an NLS peptide [26••]. The *arm* repeat domain of importin α forms a superhelical rod-like structure with each repeat folding into a three-helix bundle. The packing arrangement of the individual repeat bundles forms a surface groove that accommodates the cargo NLS in an extended β-strand conformation. Recognition of the NLS by hydrophobic and electrostatic contacts between amino acids in the NLS and in the groove of the importin *arm* repeat structure provides the basis for selectivity and strength of the interaction. Biochemical evidence supports the structural model and suggests that the organization of the repeats determines the specificity of each importin α [22,25•].

It appeared initially that the requirement for an adapter like importin α or Snurportin (see below) distinguished the importin β1 pathway from other nuclear transport pathways in which the transporters bind cargo directly. Now several proteins that bind importin β1 directly have been identified. These proteins share an arginine-rich NLS reminiscent of the IBB domain in importin α [27–29].

Snurportin and U snRNP import

The nuclear import of mature U snRNPs (small nuclear ribonucleoproteins) requires two signals: the trimethyl guanosine (m_3G) cap and the Sm core. Although not absolutely required for the import of all U snRNPs in *Xenopus* oocytes or somatic cells, the m_3G cap accelerates the kinetics of U snRNP import [2]. A role for importin β1 in the nuclear import of U snRNPs in *Xenopus* oocytes had been identified but the precise nature of the recognition of the RNP by the transport machinery was unclear [30]. This past year, Huber and coworkers [31••] provided a link between the roles of importin β1 and the m_3G cap in the import of U snRNPs with the identification of Snurportin1, a specific m_3G-cap-binding protein. Snurportin1 binds to

the m_3G cap and to importin β acting as an adapter analogous to the role of importin α in classic NLS-mediated protein import. Like importin α, Snurportin1 contains an IBB domain, however, the remaining carboxy-terminal portion of Snurportin1 has no significant homology to importin α or to any other transport factors. A database search has identified possible homologs in mouse, *Drosophila* and *Caenorhabditis elegans* suggesting evolutionary conservation of Snurportin1 function. Surprisingly, the yeast database does not contain any open reading frames with significant homology to the human protein. The nature of the NLS provided by the Sm core proteins remains to be elucidated.

The role of Ran in transport

Probably the biggest change in our thinking about the mechanism of nuclear transport revolves around the role of the small GTPase Ran and the utilization of energy in the process [4–6]. Early models of transport suggested that GTP hydrolysis by Ran directly provided the energy for transport. These models were based on observations that nonhydrolyzable analogs of GTP or mutant forms of Ran that could not efficiently hydrolyze GTP inhibited protein import. In the past couple of years, a number of studies have suggested that GTP hydrolysis by Ran does not provide the energy for transport but simply regulates the assembly and disassembly of transport complexes (as described in the introduction). Schwoebel and coworkers [32•] recently carried out a biochemical study that supports this model and which accounts for many of the confusing aspects of nucleotide utilization during transport observed in *in vitro* transport assays.

In a somewhat surprising development, three groups independently showed that importin β1, transportin or exportin-t could enter the nucleus in the absence of other transport factors, including Ran [10••,33,34]. These results suggested that individual transporters could move through the nuclear pore by energy-independent mechanisms. Could transporter–cargo complexes also move through the pore without energy or might the addition of cargo to the transporter now change the requirement for Ran? Recently, two very careful studies directly addressed the role of Ran in transporter–cargo translocation [35••,36•]. Amazingly, when single import events are observed, Ran is dispensable for some transporter–cargo combinations. Export of cargo requires Ran due to the co-operativity of Ran and cargo binding to the exportins but GTP hydrolysis is not required. Hydrolysis of GTP by Ran is apparently only required to restore the competence of the transporters for multiple rounds of transport.

NTF2 as a transporter for Ran

How does a cell maintain a concentration gradient of Ran between the nucleus and cytoplasm? Ran is small enough to diffuse readily through the nuclear pores and the numerous export pathways move a molecule of Ran from the nucleus to the cytoplasm with each export event. Combined diffusion and export would be predicted to rapidly deplete Ran from the nucleus in the absence of a counterbalancing Ran import system. Although some experiments suggest that Ran may be imported along with importin β1 [37], this pathway alone would not account for the inward movement of Ran necessary to maintain the high concentration of Ran in the nucleus. Two groups have reported recently that the Ran•GDP-binding protein NTF2 transports Ran•GDP from the cytoplasm to the nucleus [38•,39•]. They suggest that Ran sequestration in the nucleus also requires that Ran•GTP be generated from the imported Ran and that Ran•GTP associate with one of the many exportins known to accumulate in the nucleus. This model is consistent with the high affinity of NTF2 for Ran•GDP and low affinity for Ran•GTP, the direct interaction of NTF2 with nucleoporins and the ability of overexpression of Ran to overcome the loss of NTF2 in yeast (reviewed in [6]). NTF2 may have additional functions in transport as it interacts with other import factors and has recently been shown to act as a guanine nucleotide dissociation inhibitor (GDI) [40]. Microinjection of NTF2 into *Xenopus* oocytes regulates the functional size of the nuclear pore during oogenesis, suggesting that either Ran or NTF2 might play a role in pore complex structure or dynamics during transport [41].

Conclusions and future perspectives

Pathways for most of the yeast transporters have now been identified. Lagging behind is the identification of the cognate pathways in higher eukaryotes, a task that may be more difficult due to possible evolutionary divergence in transporters or localization sequences. The greatest problem facing us is to understand how transporters interact with the NPC during translocation and progress has begun to be made in this area. A number of lines of evidence suggest that some pathways may overlap in the pore but just how extensively pathways through the pore are shared is unknown. We are also gaining a better understanding of how transport works on a mechanistic level with increasingly fine characterization of specific protein–protein interactions. Further characterization of some of the accessory proteins of the transport pathway, such as RanBP1 and NTF2, will undoubtedly provide insights into the roles of both the transporters and pore complex components in transport. A big question remaining is just how energy is utilized in transport. Is the energy derived from transporter recycling used to drive import or export pathways against a concentration gradient? Do the changes in pore conformation during transport of large structures require an input of energy from some unknown source? Perhaps the permeabilized cell assay has uncoupled some energy-requiring step in transport that can only be studied in a more intact system. Now that many transport factors and pathways have been identified, the challenges are to understand their paths through the pore complex and how they are regulated.

Acknowledgements

I would like to thank everyone who contributed reprints or communicated articles in press. Thanks to Ken Geles and Gina Visser for helpful comments and discussions on the manuscript. My apologies to everyone whose work I was not able to include due to space limitations.

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Imamoto N, Kamei Y, Yoneda Y: **Nuclear transport factors: function, behavior and interaction.** *Eur J Histochem* 1998, **42**:9-20.

2. Izaurralde E, Adam S: **Transport of macromolecules between the nucleus and the cytoplasm.** *RNA* 1998, **4**:351-364.

3. Wozniak RW, Rout MP, Aitchison JD: **Karyopherins and kissing cousins.** *Trends Cell Biol* 1998, **8**:184-188.

4. Cole CN, Hammell CM: **Nucleocytoplasmic transport: driving and directing transport.** *Curr Biol* 1998, **8**:R368-R372.

5. Dasso M, Pu RT: **Nuclear transport: run by Ran?** *Am J Hum Genet* 1998, **63**:311-316.

6. Moore MS: **Ran and nuclear transport.** *J Biol Chem* 1998, **273**:22857-22860.

7. Jakel S, Görlich D: **Importin β, transportin, RanBP5 and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells.** *EMBO J* 1998, **17**:4491-4502.

8. Rosenblum JS, Pemberton LF, Bonifaci N, Blobel G: **Nuclear import and the evolution of a multifunctional RNA-binding protein.** *J Cell Biol* 1998, **143**:887-899.

9. •• Arts G-J, Fornerod M, Mattaj IW: **Identification of a nuclear export receptor for tRNA.** *Curr Biol* 1998, **8**:305-314.
Along with the following reference (Kutay *et al.* [10••]), the exportin for tRNA, exportin-t, is identified as a previously uncharacterized member of the transporter family. It stimulates the export of tRNA from micro-injected *Xenopus* nuclei, shuttles between the nucleus and cytoplasm and binds tRNA in a Ran•GTP-dependent manner. This transporter is the first shown to bind a nucleic acid rather than a protein transport signal.

10. •• Kutay U, Lipowsky G, Izaurralde E, Bischoff FR, Schwarzmaier P, Hartmann E, Görlich D: **Identification of a tRNA-specific nuclear export receptor.** *Mol Cell* 1998, **1**:359-369.
See annotation Arts *et al.* [9••].

11. • Hellmuth K, Lau DM, Bischoff LR, Kunzler M, Hurt E, Simos G: **Yeast Los1p has properties of an exportin-like nucleocytoplasmic transport factor for tRNA.** *Mol Cell Biol* 1998, **18**:6374-6386.
Subsequent to identification of the human exportin-t, Los1p protein is shown to be the yeast homolog. Los1p had been known to have an undefined role tRNA metabolism.

12. Sarkar S, Hopper AK: **tRNA nuclear export in *Saccharomyces cerevisiae*: *in situ* hybridization analysis.** *Mol Biol Cell* 1998, **9**:3041-3055.

13. Hurt DJ, Wang SS, Li Y, Hopper AK: **Cloning and characterization of *LOS1* a *Saccharomyces cerevisiae* gene that affects tRNA splicing.** *Mol Cell Biol* 1987, **7**:1208-1216.

14. • Kaffman A, Miller Rank N, O'Neill EM, Huang LS, O'Shea EK: **The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus.** *Nature* 1998, **396**:482-486.
The localization of yeast transcription factor Pho4p is affected by phosphate availability. Here it is shown that Pho4p export from the nucleus is mediated by the transporter homolog Msn5p. The interaction of Pho4p with Msn5p requires Ran•GTP and phosphorylation of Pho4p. This is the first example of an NES regulated directly by phosphorylation.

15. Jans DA: **The regulation of protein transport to the nucleus by phosphorylation.** *Biochem J* 1995, **311**:705-716.

16. Ferrigno P, Posas F, Koepp D, Saito H, Silver PA: **Regulated nucleo/cytoplasmic exchange of HOG1 MAPK requires the importin β homologs NMD5 and XPO1.** *EMBO J* 1998, **17**:5606-5614.

17. Hood JK, Silver PA: **Cse1p is required for export of Srp1p/Importin-α from the nucleus in *Saccharomyces cerevisiae*.** *J Biol Chem* 1998, **273**:35142-35146.

18. Solsbacher J, Maurer P, Bischoff FR, Schlenstedt G: **Cse1p is involved in export of yeast importin α from the nucleus.** *Mol Cell Biol* 1998, **18**:6805-6815.

19. Kamei Y, Yuba S, Nakayama T, Yoneda Y: **Three distinct classes of the α-subunit of the nuclear pore-targeting complex (Importin-α) are differentially expressed in adult mouse tissues.** *J Histochem Cytochem* 1999, **47**:1-10.

20. Nachury M, Ryder UW, Lamond AI, Weis K: **Cloning and characterization of hSRP1γ, a tissue-specific nuclear transport factor.** *Proc Natl Acad Sci USA* 1998, **95**:582-587.

21. Nadler SG, Tritschler D, Haffar OK, Blake J, Bruce AG, Cleaveland JS: **Differential expression and sequence-specific interaction of karyopherin α with nuclear localization sequences.** *J Biol Chem* 1997, **272**:4310-4315.

22. Prieve MG, Guttridge KL, Mungia J, Waterman ML: **Differential importin-α recognition and nuclear transport by nuclear localization signals within the high-mobility-group DNA binding domains of lymphoid enhancer factor 1 and T-cell factor 1.** *Mol Cell Biol* 1998, **18**:4819-4832.

23. Görlich D, Henklein P, Laskey RA, Hartmann E: **A 41 amino acid motif in importin-α confers binding to importin-β and hence transit into the nucleus.** *EMBO J* 1996, **15**:1810-1817.

24. Weis K, Ryder U, Lamond AI: **The conserved amino-terminal domain of hSRP1α is essential for nuclear protein import.** *EMBO J* 1996, **15**:1818-1825.

25. • Herold A, Truant R, Wiegand H, Cullen BR: **Determination of the functional domain organization of the importin α nuclear import factor.** *J Cell Biol* 1998, **143**:309-318.
Although it was the first transport factor identified, the functional domain structure of importin α had not been carefully dissected. A carboxy-terminal segment of the protein including a conserved acidic region binds to CAS for export of importin α from the nucleus. Two different NLSs are shown to bind to distinct subsets of the importin α *arm* repeats with minimal overlap suggesting that a single importin α protein may bind multiple NLSs.

26. •• Conti E, Uy M, Leighton L, Blobel G, Kuriyan J: **Crystallographic analysis of the recognition of a nuclear localization signal by the nuclear import factor karyopherin.** *Cell* 1998, **94**:193-204.
The crystal structure of the *arm* repeat region of importin α was determined with and without a bound peptide containing a monopartite NLS. The structure is similar to that recently described for β-catenin. Each *arm* repeat is folded into a three-helix structure and together the 10 repeats are organized into a superhelical rod. The position of amino acid residues within the structure reveals the basis for specificity of NLS binding for monopartite and bipartite NLSs. These structural studies could pave the way for the design of specific inhibitors of NLS–receptor interactions.

27. Palmeri D, Malim MH: **Importin β can mediate the nuclear import of an arginine-rich nuclear localization signal in the absence of importin α.** *Mol Cell Biol* 1999, **19**:1218-1225.

28. Truant R, Cullen BR: **The arginine-rich domains present in human immunodeficiency virus type 1 Tat and Rev function as direct importin β-dependent nuclear localization signals.** *Mol Cell Biol* 1999, **19**:1210-1217.

29. Truant R, Fridell RA, Benson RE, Bogerd H, Cullen BR: **Identification and functional characterization of a novel nuclear localization signal present in the yeast Nab2 poly(A)$^+$ RNA binding protein.** *Mol Cell Biol* 1998, **18**:1449-1458.

30. Palacios I, Hetzer M, Adam SA, Mattaj IW: **Nuclear import of U snRNPs requires importin β.** *EMBO J* 1997, **16**:6783-6792.

31. •• Huber J, Cronshagen U, Kadokura M, Marshallsay C, Wada T, Sekine M, Luhrmann R: **Snurportin 1, an m_3G-cap-specific nuclear import receptor with a novel domain structure.** *EMBO J* 1998, **17**:4114-4128.
A m_3G-cap-binding protein called snurportin1 was identified. The protein enhances cap-dependent nuclear import of U snRNPs in permeabilized cells, functioning as an snRNP import receptor. The 45 kDa protein contains an IBB domain and interacts with importin β, but has no structural similarity to other transport factors. Snurportin1 can be thought of as an adapter like importin α.

32. • Schwoebel ED, Talcott B, Cushman I, Moore MS: **Ran-independent signal-mediated nuclear import does not require GTP hydrolysis by Ran.** *J Biol Chem* 1998, **273**:35170-35175.
Nucleotide hydrolysis has long been thought of as the source of energy for nuclear transport. Although several indirect studies have recently hinted that this is not so, this study carefully biochemically characterizes the permeabilized cell assay used to study import and export. They conclude that hydrol-

ysis of NTPs is not required for protein import. The mechanism of inhibition by nonhydrolyzable nucleotide analogs in the assay is shown to depend on the presence of RCC1.

33. Kose S, Imamoto N, Tachibana T, Shimamoto T, Yoneda Y: **Ran-unassisted nuclear migration of a 97-kD component of nuclear pore-targeting complex.** *J Cell Biol* 1997, **139**:841-849.

34. Nakielny S, Dreyfuss G: **Import and export of the nuclear protein import receptor transportin by a mechanism independent of GTP hydrolysis.** *Curr Biol* 1997, **8**:89-95.

35. •• Englemeier L, Olivo J-C, Mattaj IW: **Receptor-mediated substrate translocation through the nuclear pore complex without nucleotide triphosphate hydrolysis.** *Curr Biol* 1999, **9**:30-41.
The translocation of Crm1 (Exportin1)-substrate complexes is shown to not require NTP hydrolysis in permeabilized cells. Careful analysis of the nucleotide and Ran remaining in the permeabilized cells is done to support the conclusions. Single event transportin-mediated import in the assay does not require hydrolysis of NTPs or even the presence of Ran (see also [36•]).

36. • Ribbeck K, Kutay U, Paraskeva E, Görlich D: **The translocation of transportin–cargo complexes through nuclear pores is independent of both Ran and energy.** *Curr Biol* 1999, **9**:47-50.
As in Englemeier *et al.* [35••], neither hydrolysis of nucleotide nor Ran are required for transportin-mediated protein import in permeabilized cells. A snurportin1–importin β complex also can translocate through the pore independent of Ran and energy.

37. Chi NC, Adam EJH, Adam SA: **Different binding domains for Ran-GTP and Ran-GDP/RanBP1 on nuclear import factor p97.** *J Biol Chem* 1997, **272**:6818-6822.

38. • Ribbeck K, Lipowsky G, Kent HM, Stewart M, Görlich D: **NTF2 mediates nuclear import of Ran.** *EMBO J* 1998, **17**:6587-6598.
The concentration of Ran in the nucleus is mediated by NTF2. NTF2 binds Ran•GDP in the cytoplasm and interacts with the NPC to move Ran into the nucleus. NTF2 mutants that cannot bind Ran are unable to concentrate Ran in the nucleus. Once in the nucleus, nucleotide exchange and binding to a transporter are required to retain Ran inside (see also Smith *et al.* [39•]). These two studies provide a concrete function for a somewhat enigmatic transport factor. A role for NTF2 for Ran import *in vitro* explains why not all groups find that it has an effect in the permeabilized cell assay.

39. • Smith A, Brownawell A, Macara IG: **Nuclear import of Ran is mediated by the transport factor NTF2.** *Curr Biol* 1998, **8**:1403-1406.
See annotation Ribbeck *et al.* [38•].

40. Yamada M, Tachibana T, Imamoto N, Yoneda Y: **Nuclear transport factor p10/NTF2 functions as a Ran/GDP dissociation inhibitor (Ran-GDI).** *Curr Biol* 1998, **8**:1339-1342.

41. Feldherr C, Akin D, Moore MS: **The nuclear import factor p10 regulates the functional size of the nuclear pore complex during oogenesis.** *J Cell Sci* 1998, **111**:1889-1896.