CELL STRUCTURE AND FUNCTION 25: 217–225 (2000)
© 2000 by Japan Society for Cell Biology
REVIEW

Insights into the Molecular Mechanism of Nuclear Trafficking Using Nuclear Transport Factor 2 (NTF2)

Murray Stewart

Medical Research Council Laboratory of Molecular Biology, Hills Rd., Cambridge CB2 2QH, England

ABSTRACT. **Nuclear transport factor 2 (NTF2) mediates the nuclear import of RanGDP. The simplicity and specialization of this system, combined with the availability of crystal structures of NTF2, RanGDP and their complex, has facilitated the investigation of the molecular mechanism of its trafficking. NTF2 binds to both RanGDP and FxFG repeat-containing nucleoporins. Mutants engineered on the basis of structural information together with determination of binding constants have been used to dissect the roles of these interactions in transport. Thus, NTF2 binds to RanGDP sufficiently strongly for the complex to remain intact during transport through NPCs, but the interaction between NTF2 and FxFG nucleoporins is much more transient, which would enable NTF2 to move through the NPC by hopping from one repeat to another. An analogous nucleoporin hopping mechanism may also be used by carrier molecules of the importin-β family to move through NPCs.**

Key words: nuclear trafficking/nucleoporins/structure/nuclear pores

The trafficking of macromolecules, ions and small molecules between the cytoplasm and nucleus is fundamental to eukaryotic cells (reviewed by Nakielny and Dreyfuss, 1999; Görlich and Kutay, 1999) and is mediated by nuclear pore complexes (NPCs), cylindrical proteinaceous structures, about 1,200 Å in diameter and 700 Å thick (Fig. 1), that perforate the nuclear envelope (reviewed by Stoffler *et al*., 1999; Yang *et al*., 1998). Thus, nuclear proteins such as histones and transcription factors synthesized in the cytoplasm need to be imported into the nucleus whereas mRNA, tRNA and rRNA need to be exported to the cytoplasm. Proteins are transported actively along the central axis of the NPC (Feldherr *et al*., 1984) if they contain an appropriate signal. For example, proteins containing a nuclear localization sequence (NLS) are imported into the nucleus, whereas proteins containing a nuclear export sequence (NES) are exported to the cytoplasm. Although there are several different nuclear trafficking pathways, they share a number of common features (reviewed by Nakielny and Dreyfuss, 1999; Görlich and Kutay, 1999; Stewart and Rhodes, 1999). In each, the substrate or cargo does not interact directly with the NPC, but instead is transported bound to a soluble carrier molecule, which then has to be recycled back to the original compartment. For example, as illustrated in Figure 2A, proteins containing a NLS first attach to soluble receptors of the importin-β/karyopherin-β family, either directly (Pollard *et al*., 1996) or via an adapter such as importin-α (Görlich *et al*., 1994). The importin-cargo complex then docks at the cytoplasmic face of the NPC, before being translocated through the NPC into the nucleus. Translocation is followed by displacement of the cargo from the carrier by nuclear RanGTP, after which the carrier, complexed with RanGTP, is recycled back through the NPCs to the cytoplasm where RanBP1 and RanGAP act to dissociate the complex and promote hydrolysis of the GTP bound to Ran (Bischoff and Görlich, 1997; Floer and Blobel, 1997; Lounsbury and Macara, 1997). Analogous pathways have been identified for the export of RNA and protein from the nucleus (reviewed by Nakielny and Dreyfuss, 1999; Görlich and Kutay, 1999). The interaction of carriers with their cargo is orchestrated by the nucleotide state of the Ras-family GTPase Ran (reviewed by Görlich, 1998; Melchior and Gerace, 1998), although the nuclear import of Ran itself is mediated by NTF2 (Ribbeck *et al*., 1998; Smith *et al*., 1998). There is now considerable information available on the structures of many of the soluble components involved with nuclear trafficking (reviewed by Chook *et al*., 1999) and on the way Ran functions to alter interactions between

Medical Research Council Laboratory of Molecular Biology, Hills Rd., Cambriadge CB2 2QH, England.
Tel: +44–1223–402463, Fax: +44–1223–213556
E-mail: MS@MRC-LMB.CAM.AC.UK

Abbreviations: NPC, nuclear pore complex; NTF2, nuclear transport factor 2; NLS, nuclear localization sequence; NES, nuclear export sequence; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GDI, guanine nucleotide dissociation inhibitor; WGA, wheat germ agglutinin.

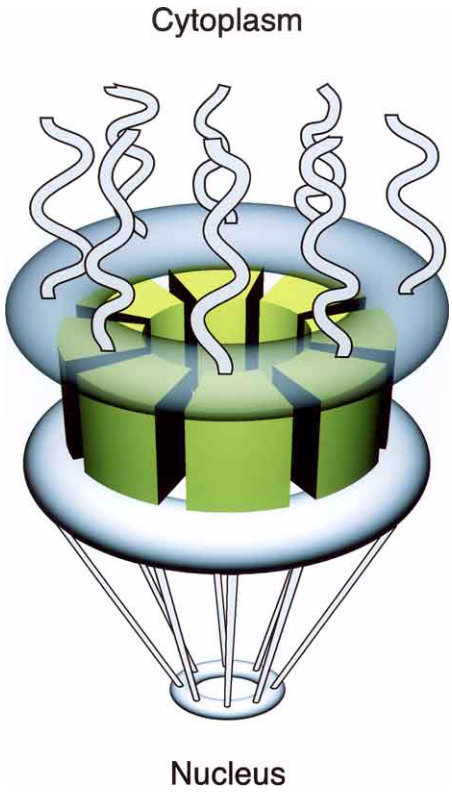

Fig. 1. Schematic drawing of a vertebrate NPC which is constructed from a central cylinder built up from eight wedge-shaped "spokes" that is sandwiched between cytoplasmic and nucleoplasmic rings. Fibres emanate from both the cytoplasmic surface of the NPC while a basket-like structure is formed on its nuclear face. Macromolecules are translocated through the NPC along a central channel.

carrier and cargo molecules in both the nucleus and the cytoplasm (reviewed by Melchior and Gerace, 1998; Görlich and Kutay, 1999; Nakielny and Dreyfuss, 1999), but less is known about the precise mechanism by which carrier-cargo complexes are translocated through the NPCs. However, the simplicity and specialization of the NTF2-mediated nuclear import of RanGDP can be exploited to give insights into how material can be translocated thought NPCs and suggests this can be achieved by NTF2 hopping between repeat-containing proteins that line the NPC transport channel.

Trafficking through nuclear pore complexes

The morphology of both yeast and vertebrate NPCs has been reviewed extensively elsewhere (Stoffler *et al*., 1999; Yang *et al*., 1998). As illustrated schematically in Figure 1, vertebrate NPCs are constructed from an approximately cylindrical central body sandwiched between nucleoplasmic and cytoplasmic rings. The central body has prominent 8-fold rotational symmetry and is constructed from eight spoke-like segments. In addition, fibres extend into both the cytoplasm and the nucleus and, in the case of the nuclear fibres, form a basket-like structure below the body of the NPC. The body of the NPC has a central channel through which macromolecules are transported, although the precise details of this feature are controversial (reviewed by Stoffler *et al*., 1999).

NPCs are constructed from multiple copies of a relatively small number of proteins and, for example, MALDI-TOF mass spectroscopy (Rout *et al*., 2000) indicates that isolated yeast NPCs contain only 40 different proteins, present mainly in either 8 or multiples of 8 copies per NPC. Moreover, there is a level of redundancy such that many *S. cerevisiae* nucleoporin nulls are viable, albeit they frequently show synthetic lethality with other nucleoporins (Doye and Hurt, 1995). Vertebrate NPCs are thought to contain additional proteins, but still probably are constructed from the order of 50–70 different proteins. NPC proteins, called collectively nucleoporins, frequently contain characteristic sequence motifs and many have similar biochemical properties. Some nucleoporins contain motifs such as those for coiled-coils that may be used as scaffolds in forming complexes (Buss and Stewart, 1995; Doye and Hurt, 1997), integral membrane domains (Rout and Wente, 1994) and Zn-finger motifs (Sukegawa and Blobel, 1993; Nakielny *et al*., 1999). However, in addition to these motifs, many nucleoporins contain characteristic FG sequence repeats, large regions consisting of tandem repeats of a hydrophobic core and a hydrophilic linker (Rout and Wente, 1994; Doye and Hurt, 1997; Rout *et al*., 2000). FG-repeats are based on highly conserved cores, containing one or two phenylalanines linked by hydrophilic spacers of variable sequence but which are rich in charged and polar residues. The two most common repeats are based on GLFG or FxFG cores (where x is usually a small residue such as serine, glycine or alanine) and nucleoporins often contain many copies of these repeats. These repeats appear to be directly involved in nuclear trafficking (reviewed by Bayliss *et al*., 2000). For example, many vertebrate FxFG nucleoporins bind wheat germ agglutinin (WGA). Nuclei assembled from *Xenopus* egg extract depleted of WGA-binding proteins do not retain the ability to import NLS-containing substrates, but this ability is restored by adding back material eluted from WGA-sepharose (Finlay and Forbes, 1990), indicating that either the WGA-binding FxFG nucleoporins, or proteins bound to them, are required for transport. Also, nuclear trafficking is blocked by antibodies that recognize FG repeats or by added nucleoporin repeat constructs (Powers *et al*., 1997; Bastos *et al*., 1996). However, different transport substrates appear to be transported via different routes using particular subsets of nucleoporins (Powers *et al*., 1997; Shah and Forbes, 1998) so that, although many nucleoporins are common to several trafficking pathways, others are only required for a specific pathway (Damelin and Silver, 2000).

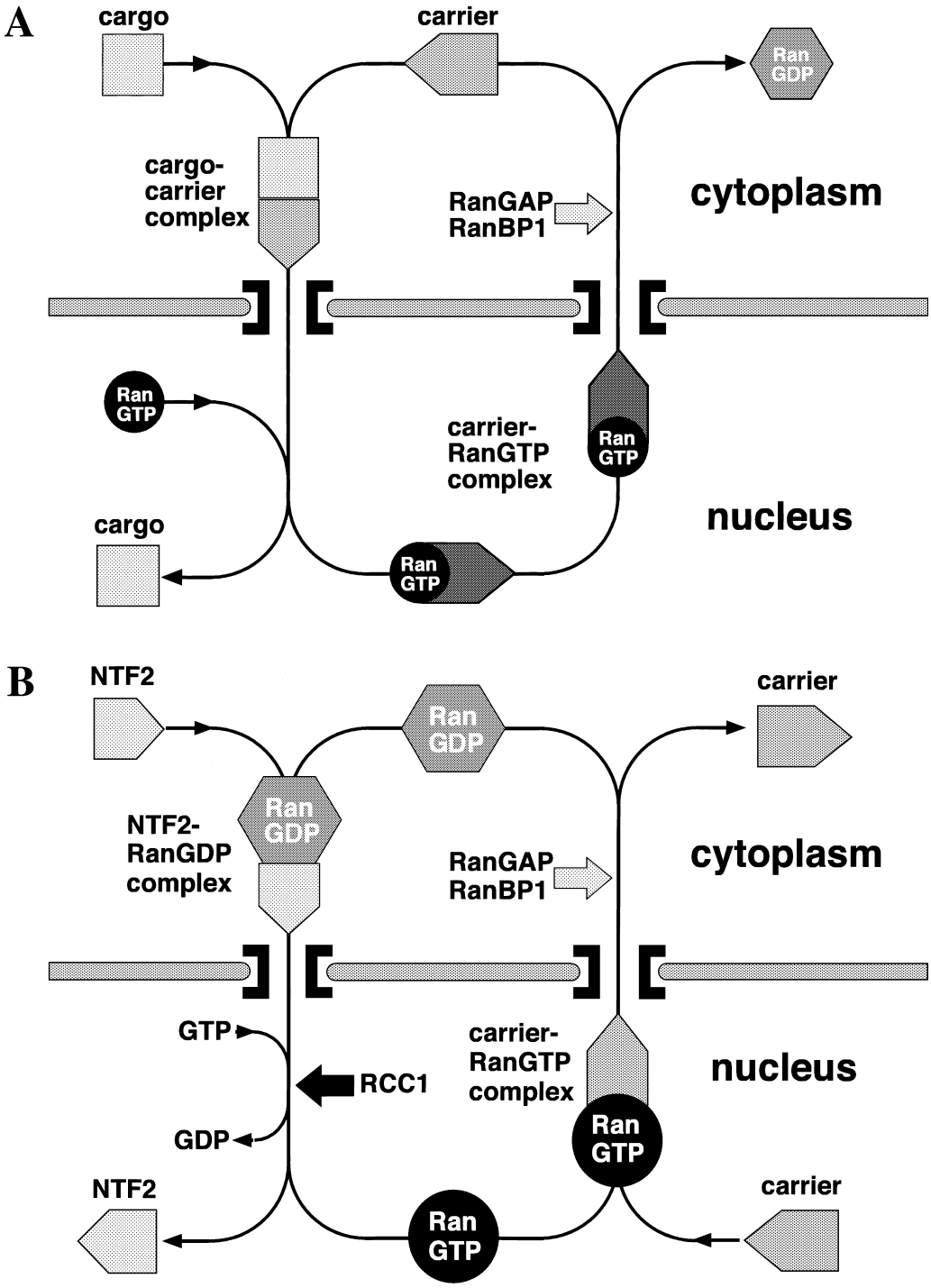

Fig. 2. (A) Simplified view of nuclear import illustrating how cargo and carrier first bind in the cytoplasm before being translocated through the NPC and are then dissociated in the nucleus by RanGTP (reproduced from Bayliss *et al*., 2000, *Traffic*, **1**: 448–456). The carrier-RanGTP complex is then recycled to the cytoplasm to participate in further rounds of transport. (B) The role of NTF2 in mediating the nuclear import of RanGDP. NTF2 binds RanGDP in the cytoplasm and mediates its nuclear import where RCC1 recharges Ran with GTP. The RanGTP is then exported to the cytoplasm attached to carrier molecules where its nucleotide is hydrolyzed to GDP by RanGAP1 in conjunction with RanBP1.

Electron microscopy has indicated that FxFG nucleoporins are located in the central channel of NPCs and also on both their nucleoplasmic and cytoplasmic faces (Grote *et al*., 1995; Stoffler *et al*., 1999; Rout *et al*., 2000). The majority of yeast nucleoporins are located symmetrically on the nuclear and cytoplasmic faces of NPC with only a few being restricted to one side. For example, in yeast, Nup60p and Nup1p are exclusively nucleoplasmic, whereas Nup159p and Nup42p are exclusively cytoplasmic (Rout *et al*., 2000). Although the localization of many nucleoporins is somewhat diffuse, some are clearly further away from the plane of the nuclear envelope than others, whereas others are on the nuclear basket or cytoplasmic fibrils rather than in the body of the NPC. Also, some labels are seen at higher radii than others. NTF2, which binds to FxFG repeats, binds to the central channel as well as the nuclear and cytoplasmic faces of NPCs when microinjected into *Xenopus* oocytes (Feldherr *et al*., 1998) or when visualized in HL60 cells using immunogold labeling (Iborra *et al*., 2000), whereas gold conjugates of NTF2 mutants that bind FxFG repeats less strongly show reduced NPC binding and are only rarely found in the central channel (Bayliss *et al*., 2000). Overall, electron microscopy appears to be consistent with the idea that FxFG repeats line the central transport channel as well coating both faces of NPCs.

Function of Ran

The nucleotide state of Ran is important in determining its interactions with other proteins and so has a crucial role in nuclear trafficking (reviewed by Koepp and Silver, 1996; Görlich, 1998), albeit GTP hydrolysis by Ran does not appear to be linked directly to transport (Kose *et al*., 1997; Nakielny and Dreyfuss, 1998; Ribbeck *et al*., 1999; Engelmeier *et al*., 1999; Schwoebel *et al*., 1998) and instead could be used, for example, to specify directionality (see Görlich, 1998) or to sort molecules prior to transport (see Stewart, 1992; Stewart and Rhodes, 1999). Ran has low intrinsic rates of nucleotide exchange and hydrolysis (Klebe *et al*., 1995), and so its nucleotide state is determined primarily by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Thus, Ran GTPase activity is stimulated by RanGAP1 (Bischoff *et al*., 1994), which is located primarily in the cytoplasm (Hopper *et al*., 1990; Melchior *et al*., 1993), whereas the Ran GEF, RCC1 (Bischoff and Ponstingl, 1991) is primarily nuclear (Ohtsubo *et al*., 1989). This spatial separation of GAP and GEF activities is thought to result in cytoplasmic Ran being primarily in the GDP-bound state whereas nuclear Ran is primarily in the GTP-bound state. This gradient of Ran nucleotide state has been proposed to be an important determinant of the directionality of transport (Görlich *et al*, 1996; Izaurralde *et al*., 1997).

In contrast to Ras, where only the GTP-bound form is active, both nucleotide states of Ran appear to be important in nuclear trafficking: RanGDP is associated with nuclear protein import, whereas RanGTP is associated with several export pathways (reviewed by Melchior and Gerace, 1998; Görlich and Kutay, 1999). A principal function of RanGTP binding to carrier molecules is to change their affinity of their cargo (Fig. 2A). On the nucleoplasmic face of the NPC, RanGTP dissociates import carriers from their cargo thereby terminating the import process (Görlich and Kutay, 1999). RanGTP is also required for formation of export cargo-carrier complexes (Wozniak *et al*., 1998). In the cytoplasm, RanGAP and RanBP1 promote hydrolysis of RanGTP bound to a transport receptor (Bischoff and Görlich, 1997). This releases Ran from the carrier thus either preparing it for another round of nuclear import or terminating a round of nuclear export (see Fig. 2A). Cycles of export by complexes containing RanGTP together with the import by complexes which do not contain Ran deplete Ran from the nucleus. Although Ran is small enough to diffuse through the NPC, this appears not to be fast enough for efficient nuclear trafficking *in vivo*.

NTF2 in nuclear trafficking

Efficient nuclear protein import using the importin-β pathway also requires NTF2 (also called p10), a 15 kDa dimeric protein that is essential in yeast and which binds to both RanGDP and to a series of nucleoporins that contain FxFG sequence motifs (Moore and Blobel, 1994; Paschal and Gerace, 1995; Corbett and Silver, 1996; Clarkson *et al*., 1997). Depletion of cytoplasm by the vertebrate FxFG nucleoporin p62 bound to Sepharose inhibits nuclear protein import in permeabilized cells (Paschal and Gerace, 1995), but transport can be restored to normal levels by adding exogenous NTF2. Although NTF2 has no detectable affinity for RanGTP (Paschal *et al*., 1996), it binds RanGDP to form a defined complex (Moore and Blobel, 1994; Paschal and Gerace, 1995; Stewart *et al*., 1998). RanGDP and FxFG-repeat containing nucleoporins bind to NTF2 *in vitro* at different, non-overlapping sites (Clarkson *et al*., 1996, 1997). Although blot overlays (Nehrbass and Blobel, 1996) indicated that NTF2 binds to both FxFG- and GLFG-repeat containing nucleoporins, yeast two-hybrid assays (Clarkson *et al*., 1997) showed only an interaction between NTF2 and FxFG-repeat containing nucleoporins and did not detect an interaction between NTF2 and GLFG-repeat containing nucleoporins.

NTF2 exists in solution as a dimer and each chain has an unusual fold based on a bent β-sheet backed by a long α-helix (Fig. 3) that generates a pronounced hydrophobic pocket (Bullock *et al*., 1996). The structure of the NTF2-RanGDP complex (Fig. 4) has been determined by X-ray crystallography (Stewart *et al*., 1998) and indicates that the primary interaction interface is formed by the hydrophobic cavity and surrounding negatively-charged residues of NTF2 (Fig. 3) which bind the switch II loop (residues 67–78) of Ran

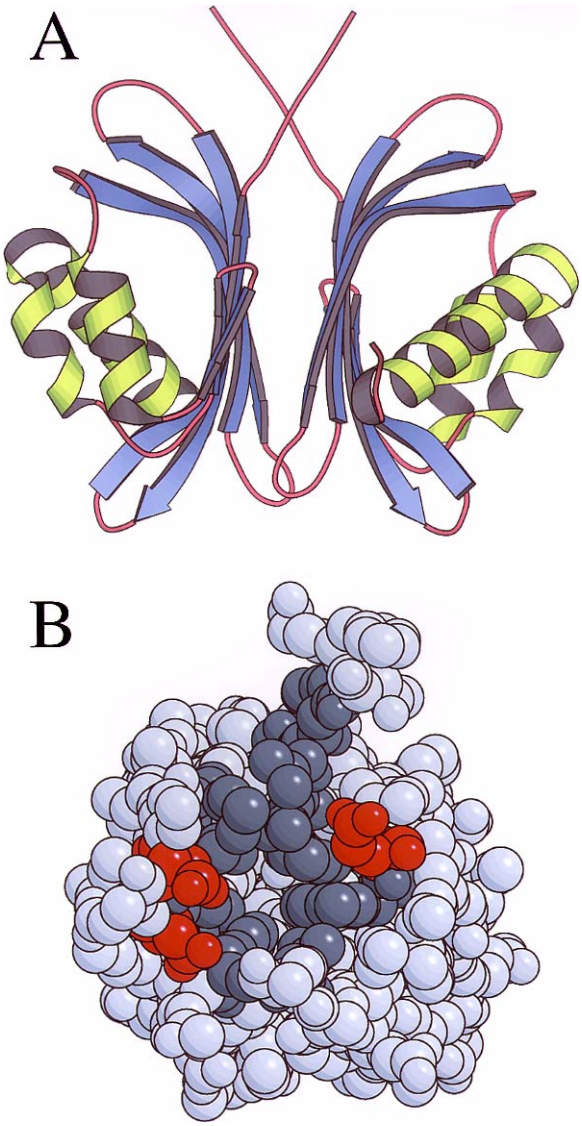

Fig. 3. (A) Crystal structure of NTF2 dimer showing how the fold of each chain is based on an unusually bent β-sheet backed by a long α-helix (Bullock *et al*., 1996). The dimer interface is based formed by the two β-sheets whereas the angled α-helix generates a distinctive hydrophobic cavity. (B) shows a space-filling model of a single NTF2 chain looking into the hydrophobic cavity. Hydrophobic residues that interact with RanGDP are grey whereas the acidic residues that form salt-bridges with Ran are red (Stewart *et al*., 1998). Figures 3–5 were generated using MolScript (Esnouf, 1997).

with a smaller contribution from the switch I region (residues 40–43). Conformational changes introduced into Ran when GTP is bound account for NTF2 binding only the GDP-bound form (Vetter *et al*., 1999). A striking feature of the interaction interface is the way in which the aromatic ring of Phe72 of Ran inserts into the hydrophobic cavity of NTF2 where it is surrounded by the hydrophobic side chains of Trp41, Leu59, Phe61, Ile64, Leu89, Ala91,

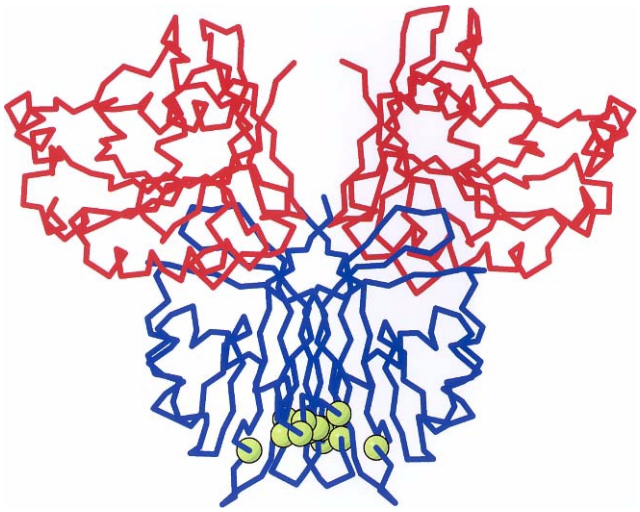

Fig. 4. Crystal structure of NTF2-RanGDP complex in which NTF2 is blue and Ran is red. The hydrophobic residues near Trp7 on NTF2 implicated in its interaction with FxFG repeats are shown as yellow spheres.

Met97, Phe119 and Leu121. This interaction accounts for 22% of the molecular surface buried by the interaction and provides a powerful explanation for why this hydrophobic residue is exposed on the surface of isolated RanGDP (Scheffzek *et al*., 1995). In addition to the residues buried in the interaction interface, there are also salt bridges between NTF2 Glu42 and Ran Arg76 and between Ran Lys71 and NTF2 Asp92 and Asp94. Although it is not possible to calculate directly the precise contributions of Ran Phe72 and the two salt bridges to the strength of the interaction between NTF2 and RanGDP, both would be expected to be substantial and disruption of the salt bridges or mutation of Phe72 is sufficient to prevent the interaction in bead-binding assays (Clarkson *et al*., 1997; Kent *et al*., 1999). The interaction between NTF2 and RanGDP is crucial for efficient nuclear protein import. Thus, mutants in either NTF2 (Clarkson *et al*., 1997) or Ran (Kent *et al*., 1999) which abolish this interaction also fail to support nuclear protein import in permeabilized cells and some temperature-sensitive Ran mutants that fail to bind to NTF2 at the non-permissive temperature (Wong *et al*., 1997) are not viable.

NTF2-mediated nuclear import of RanGDP

A key role of NTF2 is to mediate nuclear import of RanGDP (Smith *et al*., 1998; Ribbeck *et al*., 1998). Efficient nuclear import of RanGDP is essential for transport because importin β-like transport receptors exit the nucleus complexed with RanGTP and thereby continuously deplete Ran from the nucleus. As illustrated in Figure 2B, cytoplasmic NTF2 binds the RanGDP generated when RanGAP stimulates the Ran GTPase and dissociates a carrier-RanGTP complex. The NTF2-RanGDP complex is then translocated through NPCs into the nucleus where RCC1

catalyzes nucleotide exchange, recharging Ran with GTP ready for another round of transport. Mutants of NTF2 or Ran that ablate the NTF2-Ran interaction disrupt the NTF2-mediated import of RanGDP (Smith *et al*., 1998; Ribbeck *et al*., 1998) whereas overexpression of Ran can suppress NTF2 nulls in yeast (Paschal *et al*., 1997). NTF2 may function in a manner analogous to RabGDI (reviewed by Pfeffer *et al*., 1995) to direct RanGDP to the nucleus for nucleotide exchange and indeed it has been reported to show RanGDI activity (Yamada *et al*., 1998).

Interaction between NTF2 and FxFG nucleoporin repeats

In vitro binding studies have established that NTF2 interacts with FxFG nucleoporins (Paschal and Gerace, 1995; Clarkson *et al*., 1996, 1997; Chaillan-Huntington *et al*., 2000). This interaction was shown to be crucial for NTF2-mediated nuclear import of RanGDP (Bayliss *et al*., 1999) using a NTF2 mutant (W7A) engineered on the basis of structural information. The crystal structures of wild-type (Bullock *et al*., 1996) and mutant (Clarkson *et al*., 1997) NTF2 and the NTF2-RanGDP complex (Stewart *et al*., 1998) indicate that there is a hydrophobic patch on the surface of NTF2 (Fig. 5) at the opposite end of the molecule to the RanGDP binding site (Fig. 4). This patch is centred on Trp7 and mutation of this residue to Ala reduces *in vitro* binding to FxFG repeat-containing nucleoporins by at least an order of magnitude, while retaining binding to RanGDP (Bayliss *et al*., 1999). Light microscopy using fluorescein-labelled proteins indicated that W7A-NTF2 bound to nuclear envelopes more weakly than wild-type NTF2 and electron microscopy indicated that gold particles coated with W7A-NTF2 bound much less strongly to the central transport channel of the nuclear pore complexes in *Xenopus* oocytes. Moreover, in contrast to wild-type NTF2 (Smith *et al*., 1998; Ribbeck *et al*., 1998), W7A-NTF2 showed only weak stimulation of the nuclear import of fluorescently-labelled RanGDP, providing direct evidence that the mechanism for NTF2-mediated Ran import involves an interaction of NTF2 with FxFG repeat-containing nucleoporins (Bayliss *et al*., 1999).

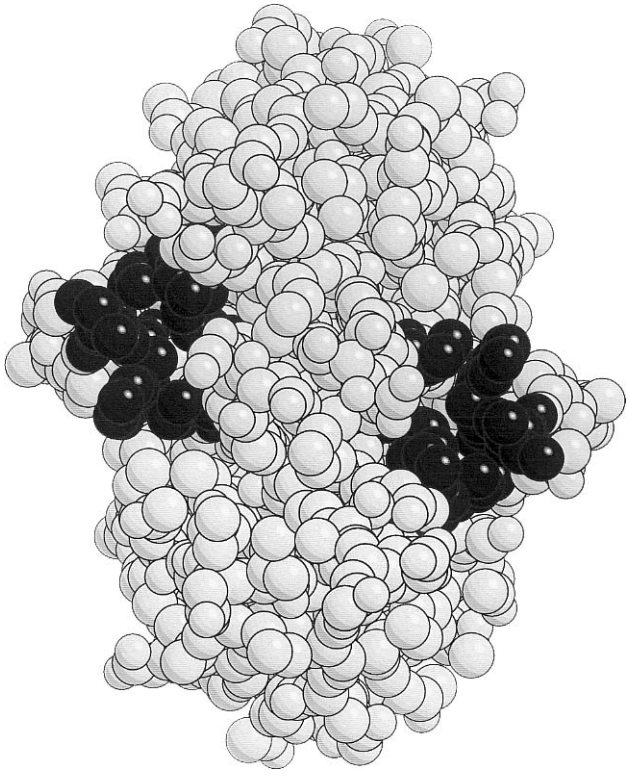

Fig. 5. Space-filling model showing the hydrophobic patch (dark grey) containing Trp7 on the surface of NTF2 implicated in the interaction with nucleoporin FxFG repeat cores. This hydrophobic patch is located on the opposite side of the molecule to the RanGDP binding site (see Figure 4).

Mechanism for trafficking

The Kd for the NTF2-RanGDP complex is of the order of 100 nM (Chaillan-Huntington *et al*., 2000) and so would be strong enough to hold the complex together during translocation through a NPC, but would allow it to dissociate quickly once in the nucleus. Binding of NTF2 to FxFG repeats is much weaker, with a Kd of the order of 25 μM (Bayliss *et al*., 1999; Chaillan-Huntington *et al*., 2000) for an individual repeat, which is consistent with NTF2 forming a number of transient attachments to FxFG nucleoporins during Ran nuclear import. The on-rate of association between NTF2 and FxFG repeats would be expected to be in the range 10^7–10^8 sec^{-1} (Berg and von Hippel, 1985), and so a 25 μM binding constant would imply an off-rate of the order of 250–2,500/sec. Consequently a binding constant of this magnitude would imply that interactions between NTF2 and FxFG nucleoporins would be transient and so ideally suited to allowing a rapid attachment and detachment of NTF2 to nucleoporins as it passed through a NPC. Such a transient interaction between NTF2 and the nucleoporins allow it to move through the NPC by jumping from one FxFG repeat to the next as illustrated schematically in Figure 6.

The results obtained with W7A-NTF2 demonstrate a direct involvement of the NTF2-FxFG nucleoporin interaction in the nuclear import of RanGDP (Bayliss *et al*., 1999) and suggest how transient interactions between NTF2 and FxFG nucleoporins could enable the NTF2-RanGDP complex to move through NPCs by hopping from one FxFG repeat to another. This direct functional interaction between FxFG nucleoporins and NTF2 raises the question of whether an analogous interaction is involved in other import pathways. Although other cargo molecules are imported using importin-β family member transport factors rather than NTF2 as carriers, there are a number of parallels between

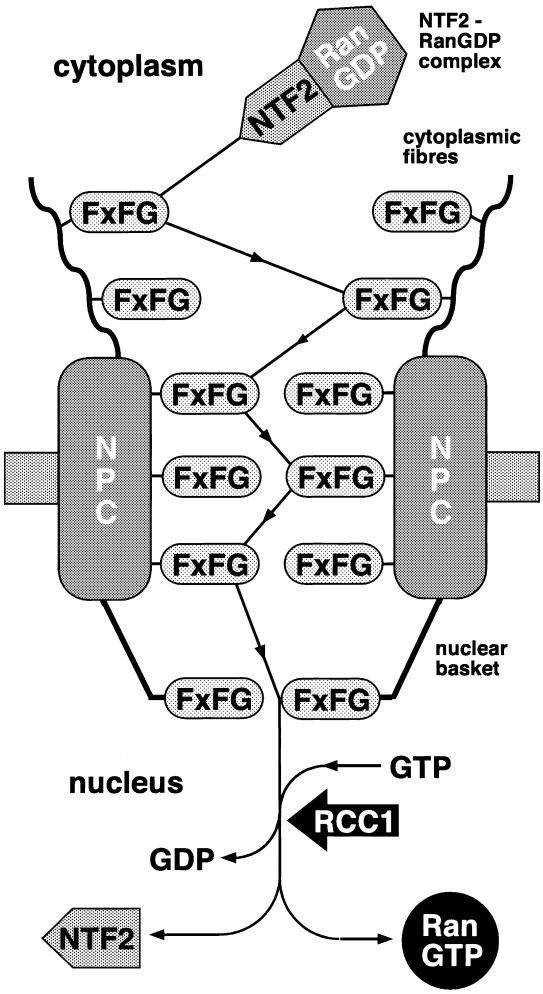

Fig. 6. Highly schematic model illustrating how NTF2 might mediate the nuclear import of RanGDP by hopping from one FxFG nucleoporin repeat to another to move through a NPC. The weak binding constant for the NTF2-FxFG nucleoporin interaction would be consistent with its forming only transient interactions with FxFG nucleoporins which would enable it to hop quickly from one to another. Because NTF2 is bound more strongly to RanGDP, this complex, by contrast, would probably remain intact during nuclear import.

the import of RanGDP and that of other proteins. For example, interactions between members of the importin-β family and several FxFG- or GLFG-repeat containing nucleoporins have been identified by both *in vitro* binding assays and the *in vivo* yeast two-hybrid system (Radu *et al*., 1995a,b; Rexach and Blobel, 1995; Bastos *et al*., 1996; Iovine *et al*., 1996; Shah *et al*., 1998). Moreover, bacterially-expressed constructs corresponding to FxFG repeat domains of several nucleoporins have been shown to exert a dominant negative effect on nucleocytoplasmic transport (Radu *et al*., 1995b; Moroianu *et al*., 1997; Shah and Forbes, 1998). A clear picture of the role of these sequence repeats in the import of carriers of the importin-β family and their cargoes has not yet emerged, although there may possibly be a correlation between interactions with GLFG nucleoporins and export (Iovine and Wente, 1997), whereas interactions with FxFG nucleoporins appear to be associated more with import (Shah and Forbes, 1998). At least for trafficking involving importin-β, Rexach and Blobel (1995) have proposed that nuclear protein import could be mediated by cycles of attachment and detachment of cargo-carrying molecules to these repeats. The extent to which the interaction of carrier molecules of the importin-β family with nucleoporins resembles that between NTF2 and nucleoporins has not been established unequivocally. Moreover, importin-β (or the importin α/β heterodimer) appears to interact more strongly with some FxFG nucleoporins than with others (see, for example, Shah *et al*., 1998), whereas NTF2 appears to interact more generally with FxFG nucleoporins (Paschal and Gerace, 1995; Clarkson *et al*., 1996, 1997). Thus, whereas NTF2 is probably binding to the FxFG cores of these repeats (Clarkson *et al*., 1997), it may be that the importin α/β heterodimer is also binding to some of the linkers. Furthermore, although addition of bacterially-expressed constructs containing the repeat regions of FxFG nucleoporins can act as a dominant negative inhibitor of nuclear protein import (Radu *et al*., 1995a; Moroianu *et al*., 1997; Shah and Forbes, 1998), it is not clear that this effect is mediated entirely through a direct interaction with importin-β. Added FxFG repeats could, for example, inhibit nuclear protein import by sequestering NTF2 and thereby inhibiting RanGDP's nuclear import and associated recharging with GTP by RCC1. Clearly further experimental work will be needed to resolve this point and, in this context, unfortunately it has not yet been possible to generate importin-β mutants analogous to W7A-NTF2 in which the binding to FxFG nucleoporins was reduced while retaining their other interactions associated with trafficking. However, the general similarity between the NTF2 and importin-β pathways certainly lends plausibility to the idea of the translocation through NPCs of an importin α/β substrate complex being mediated by a series of weak interactions with repeat-containing nucleoporins.

Questions outstanding

Although the simplicity and specialization of the NTF2-mediated nuclear import of RanGDP has given a number of insights into the molecular mechanism by which macromolecules traffic through NPCs, there are a number of questions that need to be addressed. For example, although there are a number of important similarities between the way NTF2 and importin-β interact with nucleoporins, it has not yet been possible to demonstrate that a direct FxFG-importin interaction is required for translocation. Moreover, although it has been established that a direct FxFG-NTF2 interaction is required to mediate the nuclear import of RanGDP, the precise details of this interaction and the way in which the

nucleoporin interacts with NTF2 have not yet been established. Clearly this is an important priority for X-ray crystallography or NMR. Finally, a number of NTF2 homologues have recently been reported and one of these, NXT1/P15, appears to be important for mRNA export (Black *et al*., 1999, Katahira *et al*., 1999), suggesting that perhaps the NTF2 fold is used more generally in nuclear trafficking.

Acknowledgments. I am most grateful to my colleagues in Cambridge, especially Richard Bayliss and Rosanna Baker, for their many helpful comments and criticisms. This work was supported in part by research grant RG0270 from the Human Frontiers Science Program.

References

Bastos, R., Lin, A., Enarson, M., and Burke, B. 1996. Targeting and function in mRNA export of nuclear pore complex protein Nup153. *J. Cell Biol.*, **134**: 1141–1156.

Bayliss, R., Ribbeck, K., Akin, D., Kent, H.M., Feldherr, C.M., Görlich, D., and Stewart, M. 1999. Interaction between NTF2 and xFxFG-containing nucleoporins is required to mediate nuclear import of RanGDP. *J. Mol. Biol.*, **293**: 579–593.

Bayliss, R., Corbett, A.H., and Stewart, M. 2000. The molecular mechanism of transport of macromolecules through nuclear pore complexes. *Traffic*, **1**: 448–456.

Berg, O.G and von Hippel, P.H. 1985. Diffusion controlled macromolecular interactions. *Ann. Rev. Biophys. Biophys. Chem.*, **14**: 131–160.

Bischoff, F.R. and Ponstingl, H. 1991. Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1. *Nature*, **354**: 80–82.

Bischoff, F.R., Klebe, C., Kretschmer, J., and Wittinghofer, A. 1994. RanGAP1 induces GTPase activity of nuclear Ras-related Ran. *Proc. Natl. Acad. Sci.*, **91**: 2587–2591.

Bischoff, F.R. and Görlich, D. 1997. RanBP1 is crucial for the release of RanGTP from importin beta- related nuclear transport factors. *FEBS Letters.*, **419**: 249–254.

Black, B.E., Levedque, L., Holaska, J.M., Wood, T.C., and Paschal, B.M. 1999. Identification of a NTF2-related factor that binds RanGTP and regulates nuclear protein export. *Mol. Cell. Biol.*, **19**: 8616–8624.

Bullock, T.L., Clarkson, W.D., Kent, H.M., and Stewart, M. 1996. The 1.6 Å resolution crystal structure of nuclear transport factor 2 (NTF2). *J. Mol. Biol.*, **260**: 422–431.

Buss, F. and Stewart, M. 1995. Macromolecular interactions in the nucleoporin p62 complex of rat nuclear pores: binding of nucleoporin p54 to the rod domain of p62. *J. Cell Biol.*, **128**: 251–261.

Chaillan-Huntington, C., Braslavsky, C.V., Kuhlmann, J., and Stewart, M. 2000. Dissecting the interactions between NTF2, RanGDP and the nucleoporin XFXFG repeats. *J. Biol. Chem.*, **275**: 5874–5879.

Chook, Y.M., Cingolani, G., Conti, E., Stewart, M., Vetter, I., and Wittinghofer, A. 1999. Pictures in cell biology. Structures of nuclear-transport components. *Trends Cell Biol.*, **9**: 310–311.

Clarkson, W.D., Kent, H.M., and Stewart, M. 1996. Separate binding sites on nuclear transport factor 2 (NTF2) for GDP-Ran and the phenylalanine-rich repeat regions of nucleoporins p62 and Nsp1p. *J. Mol. Biol.*, **263**: 517–524.

Clarkson, W.D., Corbett, A.H., Paschal, B.M., Kent, H.M., McCoy, A.J., Gerace, L., Silver, P.A., and Stewart, M. 1997. Nuclear protein import is decreased by engineered mutants of nuclear transport factor 2 (NTF2) that do not bind GDP Ran. *J. Mol. Biol.*, **272**: 716–730.

Corbett, A.H. and Silver, P.A. 1996. The NTF2 gene encodes an essential, highly conserved protein that functions in nuclear transport *in vivo*. *J. Biol. Chem.*, **271**: 18477–18484.

Damelin, M. and Silver, P.A. 2000. Mapping interactions between nuclear transport factors in living cells reveals pathways through the nuclear pore complex. *Mol. Cell*, **5**: 133–140.

Engelmeier, L., Olivo, J.C., and Mattaj, I.W. 1999. Receptor-mediated substrate translocation through the nuclear pore complex without nucleotide triphosphate hydrolysis. *Curr. Biol.*, **9**: 30–41.

Esnouf, R.M. 1997. An extensively modified version of MolScript that includes greatly enhanced colouring capabilities. *J. Mol. Graph. Model.*, **15**: 132–136.

Feldherr, C.M., Kallenbach, E., and Schultz, N. 1984. Movement of karyophilic protein through the nuclear pores of oocytes. *J. Cell Biol.*, **99**: 2216–2222.

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

Finlay, D.R. and Forbes, D.J. 1990. Reconstitution of biochemically altered nuclear pores: transport can be eliminated and restored. *Cell,* **60**: 17–29.

Floer, M. and Blobel, G. 1997. Disassembly of the RanGTP-Karyopherin β complex, an intermediate in nuclear protein import. *J. Biol. Chem.*, **272**: 19538–19546.

Görlich, D. 1998. Transport into and out of the cell nucleus. *EMBO J.*, **17**: 2721–2727.

Görlich, D. and Kutay, U. 1999. Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Biol.*, **15**: 607–660.

Görlich, D., Prehn, S., Laskey, R. A., and Hartmann, E. 1994. Isolation of a protein that is essential for the first step of nuclear protein import. *Cell*, **79**: 767–778.

Görlich, D., Panté, N., Kutay, U., Aebi, U., and Bischoff, F.R. 1996. Identification of different roles for RanGDP and RanGTP in nuclear protein import. *EMBO J.*, **15**: 5584–5594.

Grote, M., Kubitscheck, U., Reichelt, R., and Peters, R. 1995. Mapping of nucleoporins to the centre of the nuclear pore complex by post-embedding immunogold electron microscopy. *J. Cell Sci.*, **108**: 2963–2972.

Hopper, A.K., Traglia, H.M., and Dunst, R.W. 1990. The yeast RNA1 gene product necessary for RNA processing is located in the cytosol and apparently excluded from the nucleus. *J. Cell Biol.*, **111**: 309–321.

Iborra, F.J., Jackson, D.A., and Cook, P.R. 2000. The path of RNA through nuclear pores: apparent entry from the sides into specialized pores. *J. Cell Sci.*, **113**: 291–302.

Iovine, M.K., Watkins, J.L., and Wente, S.R. 1996. The GLFG repetitive region of the nucleoporin Nup116p interacts with Kap95p, an essential yeast nuclear import factor. *J. Cell Biol.*, **131**: 1699–1713.

Iovine, M.K. and Wente, S.R. 1997. A nuclear export signal in Kap95p is required for both recycling the import factor and interaction with the nucleoporin GLFG repeat regions of Nup116p and Nup100p. *J. Cell Biol.*, **137**: 797–811.

Izaurralde, E., Kutay, U., von Kobbe, C., Mattaj, I.W., and Görlich, D. 1997. The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. *EMBO J.*, **16**: 6535–6547.

Katahira, J., Strasser. K., Podtelejnikov, A., Mann, M., Jung, J.U., and Hurt, E. 1999. The Mex69p-mediated nuclear mRNA export pathway is conserved from yeast to human. *EMBO J.*, **18**: 2593–2609.

Kent, H. M., Moore, M.S., Quimby, B.B., Baker, A.M.E., McCoy, A.J., Murphy, G.A., Corbett, A.H., and Stewart, M. 1999. Engineered mutants in the switch II loop of Ran define the contributions made by key residues to the interaction with nuclear transport factor 2 (NTF2) and the role of this interaction in nuclear protein import. *J. Mol. Biol.*, **289**: 565–577.

Klebe, C., Prinz, H., Wittinghofer, A., and Goody, R.S. 1995. The kinetic mechanism of Ran-Nucleotide exchange catalyzed by RCC1. *Biochemistry*, **34**: 12543–12552.

Koepp, D.M. and Silver, P.A. 1996. A GTPase controlling nuclear trafficking: running the right way or walking randomly. *Cell*, **87**: 1–4.

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

Lounsbury, K.M. and Macara, I.G. 1997. Ran-binding protein 1 (RanBP1) forms a ternary complex with Ran and karyopherin β and reduces Ran GTPase-activating protein (RanGAP1) inhibition by karyopherin β. *J. Biol. Chem.*, **272**: 551–555.

Melchior, F., Weber, K., and Gerke, V. 1993. A functional homologue of the RNA1 gene product in *Schizosaccharomyces pombe*: purification, biochemical characterization, and identification of a leucine-rich repeat motif. *Mol. Biol. Cell*, **4**: 569–581.

Moore, M.S. and Blobel, G. 1994. Purification of a Ran-interacting protein that is required for protein import into the nucleus. *Proc. Natl. Acad. Sci. USA*, **91**: 10212–10216.

Moroianu, J., Blobel, G., and Radu, A. 1997. RanGTP-mediated nuclear export of karyopherin α involves its interaction with nucleoporin Nup153. *Proc. Natl. Acad. Sci. USA*, **94**: 9699–9704.

Nakielny, S. and Dreyfuss, G. 1998. Import and export of the nuclear protein import receptor transportin by a mechanism independent of GTP hydrolysis. *Curr. Biol.,* **8**: 89–95.

Nakielny, S. and Dreyfuss, G. 1999. Transport of proteins and RNAs in and out of the nucleus. *Cell*, **99**: 677–690.

Nakielny, S., Shaikh, S., Burke, B. and Dreyfuss, G. 1999. Nup153 is an M9-containing mobile nucleoporin with a novel Ran-binding domain. *EMBO J.*, **18**: 1982–1995.

Nehrbass, U. and Blobel, G. 1996. Role of the nuclear transport factor p10 in nuclear import. *Science*, **272**: 120–122.

Ohtsubo, M., Okazaki, H., and Nishimoto, T. 1989. The RCC1 protein, a regulator for the onset of chromosome condensation locates in the nucleus and binds to DNA. *J. Cell Biol.*, **109**: 1389–1397.

Paschal, B.M. and Gerace, L. 1995. Identification of NTF2, a cytosolic factor for nuclear import that interacts with nuclear pore protein p62. *J. Cell Biol.*, **129**: 925–937.

Paschal, B.M., Delphin, C. and Gerace, L. 1996. Nucleotide-specific interaction of Ran/TC4 with nuclear transport factors NTF2 and p97. *Proc. Natl. Acad. Sci. USA*, **93**: 7679–7683.

Paschal, B.M., Fritze, C., Guan, T., and Gerace, L. 1997. High levels of the GTPase Ran/TC4 relieve the requirement for nuclear protein transport factor 2. *J. Biol. Chem.*, **272**: 21534–21539.

Pfeffer, S.R., Dirac-Svejstrup, A.B., and Soldati, T. 1995. Rab GDP dissociation inhibitor: putting Rab GTPases in the right place. *J. Biol. Chem.*, **270**: 17057–17059.

Pollard, V.W., Michael, W.M., Nakielny, S., Siomi, M.C., Wang, F., and Dreyfuss, G. 1996. A novel receptor mediated nuclear protein import pathway. *Cell*, **88**: 985–994.

Radu, A., Blobel, G., and Moore, M.S. 1995a. Identification of a protein complex that is required for nuclear protein import and mediates docking of import substrate to distinct nucleoporins. *Proc. Natl. Acad. Sci., USA*, **92**: 1769–1773.

Radu, A., Moore, M.S., and Blobel, G. 1995b. The peptide repeat domain of nucleoporin Nup98 functions as a docking site in transport across the nuclear pore complex. *Cell*, **81**: 215–222.

Rexach, M. and Blobel, G. 1995. Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors and nucleoporins. *Cell*, **83**: 683–692.

Ribbeck, K., Lipowsky, G., Kent, H.M., Stewart, M., and Görlich, D. 1998. NTF2 mediates nuclear import of Ran. *EMBO J.*, **17**: 6587–6598.

Ribbeck, K., Kutay, U., Paraskeva, E., Görlich, D. 1999. The translocation of transportin-cargo complexes through nuclear pores is independent of both Ran and energy. *Curr. Biol.*, **9**: 47–50.

Rout, M.P. and Wente, S.R. 1994. Pores for thought: nuclear pore complex proteins. *Trends Cell Biol.*, **4**: 357–365.

Rout, M.P., Aitchison, J.D., Suprapto, A., Hjertaas, K., Zhao, Y., and Chait, B.T. 2000. The yeast nuclear pore complex. Composition, architecture, and transport mechanism. *J. Cell Biol.*, **148**: 635–652.

Schwoebel, E.D., Talcott, B., Cushman, I., Moore, M.S. 1998. Ran-dependent signal-mediated nuclear import does not require GTP hydrolysis by Ran. *J. Biol. Chem.*, **273**: 35170–35175.

Shah, S., Tugendreich, S., and Forbes, D. 1998. Major binding sites for the nuclear import receptor are the internal nucleoporin Nup153 and the adjacent nuclear filament protein Tpr. *J. Cell Biol.*, **141**: 31–40.

Shah, S. and Forbes, D.J. 1998. Separate nuclear import pathways converge on the nucleoporin Nup153 and can be dissected with dominant negative inhibitors. *Curr. Biol.*, **8**: 1376–1386.

Smith, A., Brownawell, A., and Macara, I.G. 1998. Nuclear import of Ran is mediated by the transport factor NTF2. *Curr. Biol.*, **8**: 1403–1406.

Stewart, M. 1992. Nuclear pore structure and function. *Seminars Cell Biol.*, **3**: 267–277.

Stewart, M. and Rhodes, D. 1999. Switching affinities in nuclear trafficking. *Nature Struct. Biol.*, **6**: 301–304.

Stewart, M., Kent, H.M., and McCoy, A.J. 1998a. Structural basis for molecular recognition between nuclear transport factor 2 (NTF2) and the GDP-bound form of the Ras-family GTPase Ran. *J. Mol. Biol.*, **277**: 635–646.

Stewart, M., Kent, H.M., and McCoy, A.J. 1998b. The structure of the Q69L mutant of GDP-Ran shows a major conformational change in the switch II loop that accounts for its failure to bind nuclear transport factor 2 (NTF2). *J. Mol. Biol.*, **284**: 1517–1527.

Stoffler, D., Fahrenkrog, B., and Aebi, U. 1999. The nuclear pore complex: from molecular architecture to functional dynamics. *Curr. Opin. Cell Biol.*, **11**: 391–401.

Vetter, I.R., Nowak, C., Nishimoto, T., Kuhlmann, J., and Wittinghofer, A. 1999. Structure of a Ran-binding domain complexed with Ran bound to a GTP analogue: implications for nuclear transport. *Nature*, **389**: 39–46.

Wong, D.H., Corbett, A.H., Kent, H.M., Stewart, M. and Silver, P.A. 1997. Interaction between the small GTPase Ran/Gsp1p and Ntf2p is required for nuclear transport. *Mol. Cell. Biol.*, **17**: 3755–3767.

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

Yang, Q., Rout, M.P., and Akey, C.W. 1998. Three-dimensional architecture of the isolated yeast nuclear pore complex: functional and evolutionary implications. *Mol. Cell*, **1**: 223–234.

(*Received for publication, April 7, 2000*

and accepted, April 7, 2000)