

Nup153 is an M9-containing mobile nucleoporin with a novel Ran-binding domain

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We employed a phage display system to search for proteins that interact with transportin 1 (TRN1), the import receptor for shuttling hnRNP proteins with an M9 nuclear localization sequence (NLS), and identified a short region within the N-terminus of the nucleoporin Nup153 which binds TRN1. Nup153 is located at the nucleoplasmic face of the nuclear pore complex (NPC), in the distal basket structure, and functions in mRNA export. We show that this Nup153 TRN1-interacting region is an M9 NLS. We found that both import and export receptors interact with several regions of Nup153, in a RanGTP-regulated fashion. RanGTP dissociates Nup153–import receptor complexes, but is required for Nup153–export receptor interactions. We also show that Nup153 is a RanGDP-binding protein, and that the interaction is mediated by the zinc finger region of Nup153. This represents a novel Ran-binding domain, which we term the zinc finger Ran-binding motif. We provide evidence that Nup153 shuttles between the nuclear and cytoplasmic faces of the NPC. The presence of an M9 shuttling domain in Nup153, together with its ability to move within the NPC and to interact with export receptors, suggests that this nucleoporin is a mobile component of the pore which carries export cargos towards the cytoplasm.

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Introduction

Transport of proteins and RNA–protein (RNP) complexes between the nucleus and the cytoplasm is essential for both constitutive and regulated cellular activities. While some proteins and RNP complexes undergo unidirectional transport across the nuclear envelope, for example import of a subset of proteins and export of mRNA, tRNA and rRNA, others undergo bidirectional trafficking in the course of their maturation and/or function (reviewed in Nakielny *et al.*, 1997; Gorlich, 1998; Izaurralde and Adam, 1998; Mattaj and Englmeier, 1998). For example, spliceosomal U snRNAs are exported, processed in the cytoplasm, and re-imported into the nucleus as mature snRNPs (Nakielny *et al.*, 1997; Mattaj and Englmeier, 1998), and many hnRNP proteins shuttle continuously in

and out of the nucleus in order to perform their probable function in the export of mRNA (reviewed in Pinol-Roma, 1997). Regulation of both unidirectional and bidirectional transport adds a further level of complexity to the nucleocytoplasmic traffic (reviewed in Cole and Saavedra, 1997; Nigg, 1997).

All cargos get into and out of the nucleus through very large proteinaceous structures termed nuclear pore complexes (NPCs) which span the nuclear envelope (reviewed in Rout and Went, 1994; Davis, 1995; Doye and Hurt, 1997). In higher eukaryotes, the NPC is ~125 MDa, and in yeast it is ~66 MDa (Doye and Hurt, 1997; Yang *et al.*, 1998). Each NPC is comprised of 50–100 different proteins, termed nucleoporins, that assemble in multiple copies to form the complex. Biochemical, genetic and genome sequencing approaches have led to the identification of ~30 yeast nucleoporins or NPC-associated proteins, while about half as many higher eukaryotic nucleoporins have been characterized. Many of the nucleoporins identified so far have one sequence motif in common, the FG (phenylalanine–glycine) repeat. This motif generally is repeated multiple times, and is found within at least two distinct sequence contexts, FXFG and GLFG, where X is an amino acid with a small or polar side chain (Rout and Went, 1994; Davis, 1995; Doye and Hurt, 1997). As yet, the function(s) of FG repeats is unknown.

Progress in characterizing the components and principles of nucleocytoplasmic transport has been rapid over the last few years. Basic tenets have been established, namely that import and export of proteins and RNP complexes are generally dictated by specific protein and/or RNA signals in the cargo which are recognized by soluble receptors (Nakielny *et al.*, 1997; Gorlich, 1998; Izaurralde and Adam, 1998; Mattaj and Englmeier, 1998). The receptors are generally large proteins (~100 kDa), and are related to each other in primary sequence (Fornerod *et al.*, 1997b; Gorlich *et al.*, 1997). They mediate association with, and translocation through, the NPC, in a process that is temperature dependent and requires the small GTPase Ran (reviewed in Rush *et al.*, 1996; Dahlberg and Lund, 1998; Melchior and Gerace, 1998). Central to one function of Ran in nucleocytoplasmic transport is the maintenance of Ran in a predominantly GTP-bound form in the nucleus, and in a GDP-bound form in the cytoplasm. This is achieved by distinct and restricted subcellular localizations of the only known guanine nucleotide exchange factor for Ran, RCC1, and the only known Ran GTPase-activating protein, RanGAP. RCC1 is a nuclear protein, while RanGAP is restricted to the cytoplasm and to the cytoplasmic side of the NPC (Matunis *et al.*, 1996; Rush *et al.*, 1996; Mahajan *et al.*, 1997; Dahlberg and Lund, 1998; Melchior and Gerace, 1998). Nuclear RanGTP dissociates import receptor–cargo complexes and therefore

functions in the release of imported cargo into the nucleoplasm (Rexach and Blobel, 1995; Gorlich *et al.*, 1996b; Izaurralde *et al.*, 1997b; Siomi *et al.*, 1997). It has exactly the opposite effect on export receptor–cargo complexes, i.e. RanGTP facilitates their formation (Fornerod *et al.*, 1997a; Kutay *et al.*, 1997a, 1998; Arts *et al.*, 1998). When the export complex reaches the cytoplasm, Ran-binding protein 1 (RanBP1) cooperates with RanGAP to effect the release of cargo from the complex with the concomitant conversion of RanGTP to RanGDP (Bischoff and Gorlich, 1997; Kutay *et al.*, 1997a, 1998). Return of RanGDP to the nucleus is mediated by the NTF2 protein (Ribbeck *et al.*, 1998). While these general principles of nucleocytoplasmic transport have been established, the detailed molecular mechanism of translocation within the NPC remains mysterious. Large proteins can be translocated in the absence of RanGTP hydrolysis and any other NTP hydrolysis (Izaurralde *et al.*, 1997b; Kose *et al.*, 1997; Nakielny and Dreyfuss, 1997; Richards *et al.*, 1997; reviewed in Dahlberg and Lund, 1998), so the driving force is not yet understood.

Several nuclear import signal–receptor systems have been described. Classical, basic nuclear localization signal (NLS)-containing proteins are imported by a dimeric complex comprising an adaptor, importin α , which binds the NLS, and the receptor, importin β , that mediates translocation through the NPC (reviewed in Nigg, 1997; Gorlich, 1998; Izaurralde and Adam, 1998; Mattaj and Englmeier, 1998). The majority of the other six or more import signal–receptor systems described so far reveal that the adaptor can be dispensed with. For example, transportin (Kap104p in yeast) is distantly related to importin β , and mediates the import of a subset of hnRNP proteins bearing an M9-type NLS that is rich in glycine and aromatic residues (Siomi and Dreyfuss, 1995; Weighardt *et al.*, 1995; Aitchison *et al.*, 1996; Pollard *et al.*, 1996; Bonifaci *et al.*, 1997; Fridell *et al.*, 1997; Siomi *et al.*, 1997, 1998; Truant *et al.*, 1998). Transportin binds directly to the M9-containing cargo (Nakielny *et al.*, 1996).

Characterization of nuclear export pathways has so far revealed four export signal–receptor systems. Exportin 1 (also known as CRM1) mediates the export of leucine-rich nuclear export signal (NES)-bearing proteins (Fornerod *et al.*, 1997a; Fukuda *et al.*, 1997; Neville *et al.*, 1997; Ossareh-Nazari *et al.*, 1997; Stade *et al.*, 1997), and exportin 2 (also known as CAS1) exports importin α , which contains a novel NES (Boche and Fanning, 1997; Kutay *et al.*, 1997a; Herold *et al.*, 1998). Nuclear export of tRNA is mediated by exportin-t which binds directly to an as yet undefined NES in tRNA (Arts *et al.*, 1998; Kutay *et al.*, 1998). The molecular mechanism of a regulated export process was recently uncovered. The export receptor Msn5 recognizes only the phosphorylated form of the transcription factor Pho4 and thereby mediates its export from the nucleus of yeast under phosphate-rich growth conditions (Kaffman *et al.*, 1998).

Towards understanding the detailed molecular mechanism of translocation through the NPC, a map revealing the localization of individual nucleoporins within the NPC is being established, and interactions of transport receptor–cargo complexes that may be relevant to the translocation process are being uncovered. Within the vertebrate NPC, RanBP2/Nup358 (Wilken *et al.*, 1995; Wu *et al.*, 1995;

Yokoyama *et al.*, 1995), CAN/Nup214/p250 and Nup84 (Kraemer *et al.*, 1994; Pante *et al.*, 1994; Bastos *et al.*, 1997; Fornerod *et al.*, 1997b) have been localized to filaments that extend from the cytoplasmic face. A complex of nucleoporins, Nup62/58/54/45, lies within the central region of the pore complex (Guan *et al.*, 1995), and Nup98 (Radu *et al.*, 1995), Nup93 (Grandi *et al.*, 1997) and Nup153 (Sukegawa and Blobel, 1993; Pante *et al.*, 1994) are components of the so-called nuclear basket that consists of filaments extending into the nucleoplasm, connected at their ends by a small ring. Finally, Tpr is localized to fibers that extend from the nuclear basket into the nucleoplasm (Cordes *et al.*, 1997). Import receptor interactions with nucleoporins are generally mediated by the nucleoporin FG repeat regions (for examples, see Iovine *et al.*, 1995; Kraemer *et al.*, 1995; Radu *et al.*, 1995; Rexach and Blobel, 1995; Hu *et al.*, 1996; Shah *et al.*, 1998).

In the course of characterizing the TRN1 import pathway, we identified a novel interaction with the nucleoporin Nup153 within a region devoid of FG repeats. Further characterization of this region of Nup153 established that it is an M9-type NLS. Consistent with the presence of an M9 shuttling domain in Nup153, we find that this nucleoporin is mobile within the NPC, as anti-Nup153 antibodies injected into the cytoplasm rapidly concentrate in the nucleus in a rim pattern. The identification of a receptor–nucleoporin interaction that is independent of FG repeats led us to examine the interactions of other import and export receptors with Nup153. We report that transport receptors can interact with several regions of Nup153. These interactions are regulated by RanGTP, which generally prevents import receptor interactions and, in contrast, promotes export receptor interactions. In addition, we have identified a novel Ran-binding domain that contains the zinc finger motif of Nup153.

Results

Transportin 1 interacts with the N-terminus of Nup153 in a Ran-regulated manner

To search for proteins that interact with TRN1, we employed a phage display screen. In this system for detecting protein–protein interactions, GST–TRN1 was immobilized on glutathione–Sephrose beads and incubated with bacteriophage T7 that display proteins expressed from a HeLa cDNA library on their surface (see Materials and methods). After four rounds of selection, 55 clones were sequenced. Sixteen of the selected clones coded for members of the hnRNP protein family, or for proteins with RNP domains, demonstrating that the screen conditions had selected effectively for cargos of TRN1 (Table I). In addition, 13 of the clones coded for ribosomal proteins. These may be non-specific interactions, or they may be bona fide TRN1 interactors, since TRN1 recently has been reported to also import ribosomal proteins *in vitro* (Jakel and Gorlich, 1998).

One clone coded for a short region (amino acids 247–290) within the N-terminus of Nup153, a component of the NPC (Table I and Figure 1A). Since this clone was selected a second time in a different phage display screen with GST–TRN1 as the bait (data not shown), we decided to characterize this interaction further.

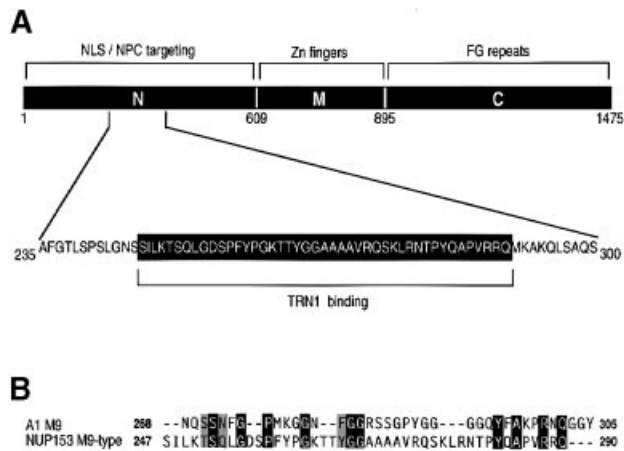


Fig. 1. Schematic representation of human Nup153, and sequence of the M9-like region. (A) Nup153 can be visualized in three domains (McMorrow *et al.*, 1994). The N-terminal (N) domain has NLS/NPC targeting activity and six FG motifs, the middle (M) domain contains four zinc finger motifs of the C₂C₂ type and two FG motifs, and the C-terminal (C) domain contains 25 FG motifs. The TRN1-binding region (amino acids 247–290), identified in the phage display screen with GST–TRN1 as bait, and the M9-like NLS (amino acids 235–300) are indicated. (B) Alignment of hnRNP A1 M9 and the Nup153 M9-like region. The alignment was performed using the ClustalW program. Identical residues are boxed in black, similar residues are boxed in gray, and the amino acid range of each sequence is indicated.

Table I. Clones selected by phage display screening with GST–TRN1 as bait

Clone	No. of times selected
hnRNP A1	4
hnRNP A2	3
FBRNP	4
TAF _{II} 68/RBP56	4
hnRNP M	1
Nup153	1
Ribosomal proteins	13

Inserts were sequenced from 55 phage that were selected after four cycles of screening. The table lists clones coding for bona fide TRN1 interactors (hnRNPA1 and A2) together with clones coding for proteins that are good candidates for real TRN1 interactors. FBRNP (fetal brain RNA-binding protein; Takiguchi *et al.*, 1993) is related to hnRNP A1, and TAF_{II}68/RBP56 (TBP-associated factor 68/RNA-binding protein 56; Bertolotti *et al.*, 1996; Morohoshi *et al.*, 1996) encodes an RNP motif protein.

Almost all transport receptor–nucleoporin interactions reported to date are restricted to the FG repeat domains of the nucleoporins (Iovine *et al.*, 1995; Kraemer *et al.*, 1995; Radu *et al.*, 1995; Rexach and Blobel, 1995; Hu *et al.*, 1996; Shah *et al.*, 1998), although p62 interacts with importin β via a coiled-coil motif (Percipalle *et al.*, 1997). The N-terminal region of Nup153 which we identified in the phage display screen is devoid of FG repeats, or indeed of any recognizable motif (Sukegawa and Blobel, 1993; McMorrow *et al.*, 1994). This, together with the fact that the conditions employed for the screen selected for TRN1 cargos, led us to test the possibility that Nup153 may be a novel cargo for the TRN1 import pathway. We confirmed the TRN1–Nup153 interaction by an alternative protein binding assay. A fusion of pyruvate kinase (PK) and Nup153[235–300] was produced by translation *in vitro* in the presence of [³⁵S]methionine, and tested for

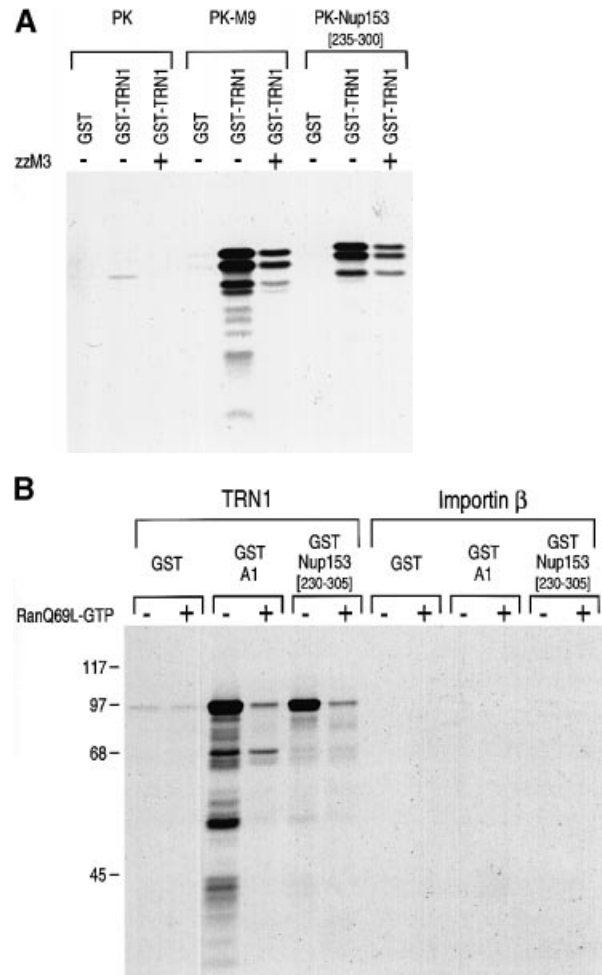


Fig. 2. (A) Nup153[235–300] binds to TRN1 *in vitro*, and the interaction is competed by the M3 NLS of A1. Pyruvate kinase (PK) fusions were translated *in vitro* in the presence of [³⁵S]methionine, and incubated with GST (2 μ g) or GST–TRN1 (2 μ g) loaded glutathione–Sepharose beads, in the absence or presence of recombinant zzm3 (15 μ g). Bound PK fusions were eluted by boiling the beads in SDS–PAGE sample buffer, and analyzed by SDS–PAGE and autoradiography. (B) Nup153[230–305] interacts specifically with TRN1 and the interaction is disrupted by RanGTP. The import receptors TRN1 and importin β were translated *in vitro* in the presence of [³⁵S]methionine, and incubated with glutathione–Sepharose beads loaded with 5 μ g of GST, GST–A1 or GST–Nup153[230–305], in the absence or presence of RanQ69L–GTP (10 μ g). Bound receptors were eluted by boiling the beads in SDS–PAGE sample buffer, and analyzed by SDS–PAGE and autoradiography.

binding to GST–TRN1 (Figure 2A). TRN1 interacted with Nup153, and the interaction was competed effectively by recombinant M3, a C-terminal portion of A1 that spans the M9 NLS (Siomi and Dreyfuss, 1995). The interaction of Nup153 with TRN1 and its competition by M3 is similar to the interaction of M9 with TRN1 (Figure 2A).

One feature of a receptor–import cargo interaction is its response to RanGTP, which binds to the receptor and triggers dissociation of the receptor–cargo complex (Rexach and Blobel, 1995; Gorlich *et al.*, 1996b; Izaurralde *et al.*, 1997b; Siomi *et al.*, 1997). RanGTP thereby serves to release cargo into the nucleoplasm. We therefore tested the effect of RanQ69L–GTP [a point mutant of Ran that cannot hydrolyze GTP at a significant rate, (Klebe *et al.*, 1995)] on the Nup153–TRN1 interaction. GST–

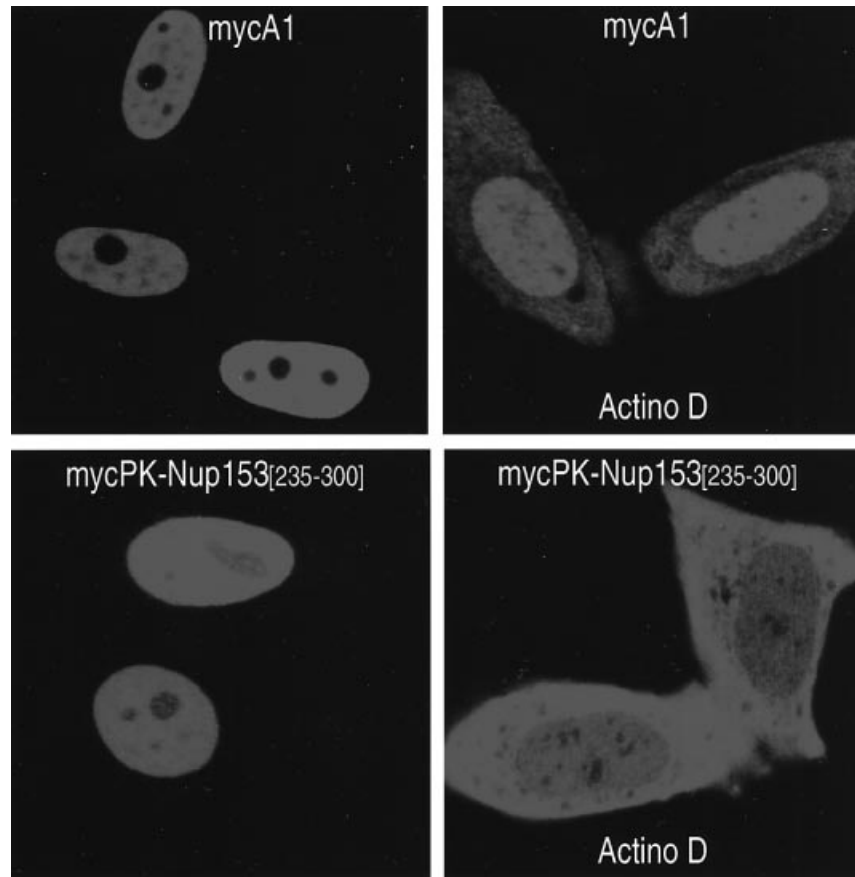


Fig. 3. Nup153[235–300] is a transcription-sensitive NLS *in vivo*. HeLa cells were transfected with DNA encoding myc-A1 or myc-PK–Nup153[235–300]. Approximately 24 h post-transfection, cells were incubated in the absence or presence of 5 μ g/ml actinomycin D for 4 h, and the subcellular localizations of the transfected proteins were analyzed by immunofluorescence microscopy using a monoclonal anti-myc antibody, 9E10.

Nup153[230–305] interacted with 35 S-labeled TRN1, and binding was almost completely abolished in the presence of RanQ69L-GTP (Figure 2B). Thus, the Nup153–TRN1 and the A1–TRN1 interaction respond identically to RanGTP. We also tested the receptor specificity of the Nup153–TRN1 interaction. Importin β does not bind to this region of Nup153 under conditions that support efficient TRN1 binding (Figure 2B).

The N-terminus of Nup153 contains a functional M9-type NLS

We tested for NLS activity of Nup153[235–300] by first fusing this portion of Nup153 to a reporter protein, PK. In cells transfected with DNA encoding PK alone, PK localizes to the cytoplasm (Siomi and Dreyfuss, 1995; Nakielny and Dreyfuss, 1996; data not shown). However, a fusion of Nup153[235–300] and PK localizes completely to the nucleus of the transfected cells (Figure 3). This region of Nup153 therefore has NLS activity *in vivo*.

An interesting property of the M9 NLS is its sensitivity to the activity of RNA polymerase II (pol II). In cells incubated with pol II inhibitors such as actinomycin D, A1 accumulates in the cytoplasm and M9 has been found to be the sensor of pol II inhibition (Pinol-Roma and Dreyfuss, 1992; Siomi *et al.*, 1997). PK–Nup153[235–300] also relocates to the cytoplasm in response to inhibition of pol II transcription (Figure 3). The relocalization of the Nup153 fusion is more dramatic than that of A1 (Pinol-Roma and Dreyfuss, 1992), and is more similar

to the response of PK-M9 to transcription inhibition (Siomi *et al.*, 1997). This is probably due to the RNA-binding activity of A1, which allows it to bind to RNA in the nucleus, or to other interactions of A1 with nuclear proteins. Intranuclear binding of full-length A1 presumably renders it less sensitive to relocalization to the cytoplasm (Siomi *et al.*, 1997).

To confirm further the conclusion that Nup153 contains an M9-type NLS, we analyzed the behavior of recombinant GST–Nup153[230–305] in the *in vitro* protein import assay. In this assay, HeLa cells are incubated with digitonin such that the cytoplasmic membrane is perforated, but the nuclear membrane remains intact (Adam *et al.*, 1990). The digitonin-permeabilized cells are washed to remove soluble cell components, and then incubated with import factors (either recombinant proteins or provided by cell extracts such as reticulocyte lysate), an energy-regenerating system and recombinant import cargo that can be detected by immunofluorescence. We analyzed the import of three cargos, GST–M9 (imported by the TRN1 pathway), GST–IBB (IBB is the importin β -binding domain of importin α , and is sufficient to direct a heterologous protein to the nucleus via the importin β pathway; Gorlich *et al.*, 1996a; Weis *et al.*, 1996), and GST–Nup153[230–305] (test cargo). In the presence of reticulocyte lysate and an energy-regenerating system, all three cargos accumulated in the nuclei of the permeabilized cells, although the import of GST–Nup153[230–305] appears less robust than that of GST–M9 and GST–IBB (Figure

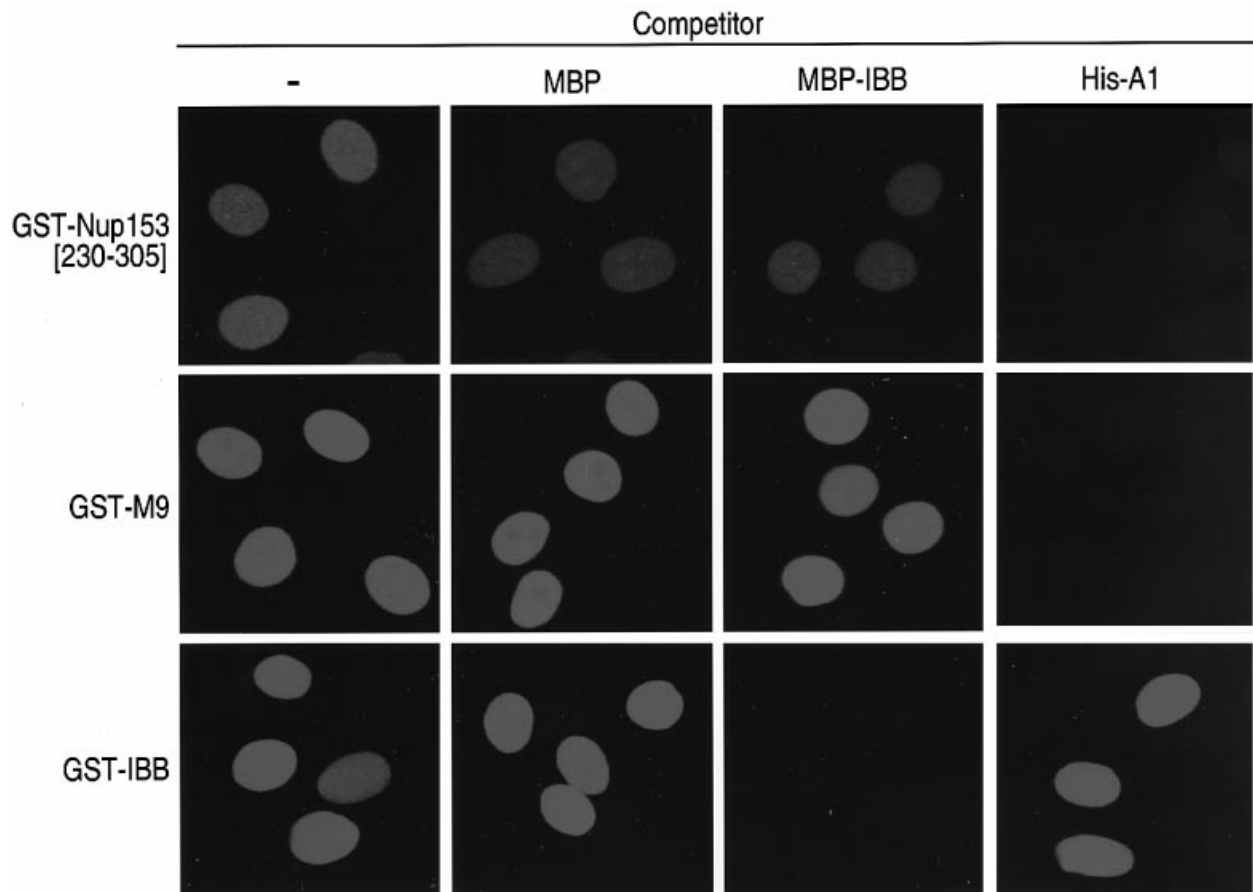


Fig. 4. Nup153[230–305] is imported by the TRN1 pathway *in vitro*. Digitonin-permeabilized HeLa cells were incubated with reticulocyte lysate, an energy-regenerating system and the indicated GST fusion protein import cargo. Import was allowed to proceed for 20 min in the absence of competitor (–), or in the presence of an ~10× molar excess (over cargo) of maltose-binding protein (MBP), MBP–IBB or His-A1. Assays were terminated by fixing the cells in formaldehyde, and the localization of the import cargo was analyzed by immunofluorescence microscopy using a monoclonal anti-GST antibody.

4, column 1). This N-terminal region of Nup153 is, therefore, a bona fide NLS that functions *in vitro* (Figure 4) as well as *in vivo* (Figure 3). To identify the specific import pathway taken by GST–Nup153[230–305], we tested the effects of competitors of the importin β pathway [maltose-binding protein (MBP)–IBB] and of the TRN1 pathway (His-A1). MBP–IBB blocks GST–IBB import, and has no effect on the import of either GST–M9 or GST–Nup153[230–305] (Figure 4, columns 2 and 3), while His-A1 abolishes the import of both GST–M9 and GST–Nup153[230–305], but has no effect on GST–IBB import (Figure 4, column 4). Altogether, the data presented in Figures 1–4 indicate that Nup153 contains a functional M9-type NLS that allows for its nuclear import by the TRN1 import pathway.

Nup153 is a mobile component of the NPC

The identification of an M9 NLS within Nup153 suggests that this domain, like A1 M9, may confer nuclear export as well as import on Nup153 (Michael *et al.*, 1995). To address this possibility, we injected an anti-Nup153 antibody, together with control IgG, into the cytoplasm of mammalian cells and monitored the localization of the antibody molecules 1 h post-injection. While the control antibody remained at the site of injection, the anti-Nup153 antibody decorated the rim

of the nucleus (Figure 5A). In contrast to the anti-Nup153 antibody, an antibody to another nuclear protein, lamin A, does not redistribute when injected into the cytoplasm (Figure 5A).

When we attempted to detect the cytoplasmically injected Nup153 antibody in cells permeabilized with digitonin (which perforates only the plasma membrane, leaving the nuclear membrane intact), the nuclear envelope-associated Nup153 antibody was not accessible to the secondary detection antibody, just as a lamin A antibody cannot detect lamin A that is localized on the nucleoplasmic side of the nuclear envelope (Figure 5B). Only when the nuclear membrane is permeabilized using Triton X-100, is the Nup153 antibody accessible to the secondary antibody (Figure 5B). The cytoplasmic Nup153 antibody is able, therefore, to traverse the NPC and accumulate at the nucleoplasmic side of the membrane. The antibody moves into the nucleus rapidly, since significant accumulation at the rim is detected as early as 20 min post-injection (data not shown). Since all microinjections were performed in the presence of cycloheximide to inhibit new protein synthesis, these observations indicate that Nup153 is exposed transiently to the cytoplasm, and are consistent with the concept that Nup153 is a mobile component that shuttles between the nuclear and cytoplasmic faces of the NPC. However, we

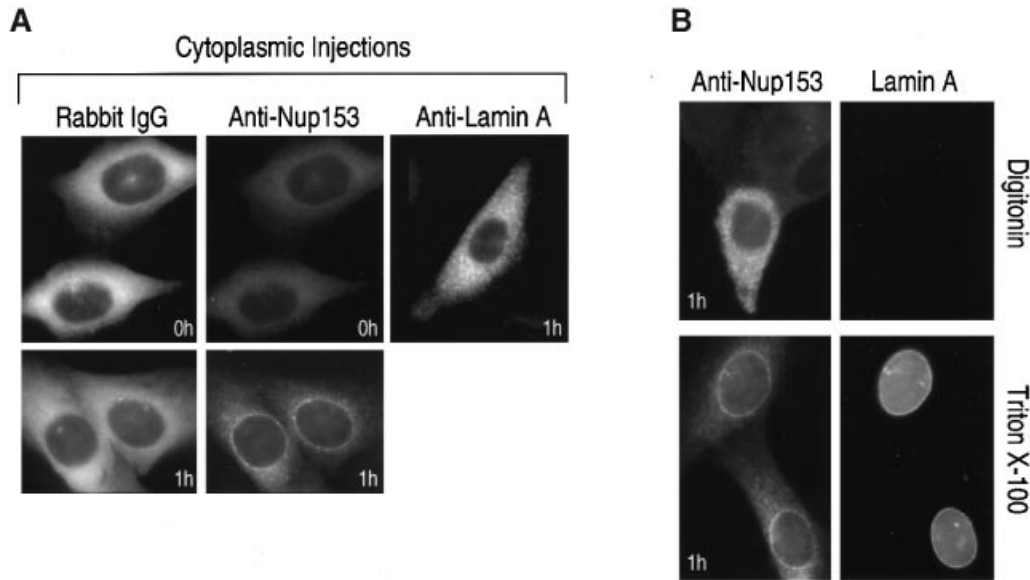


Fig. 5. Nup153 is mobile within the NPC. (A) Anti-Nup153 monoclonal antibody together with non-immune rabbit IgG were microinjected into the cytoplasm of NRK cells. In a separate experiment, anti-lamin A antibody was injected into the cytoplasm. The cells were fixed and processed for immunofluorescence microscopy either immediately after microinjection (0 h) or following a 1 h incubation at 37°C (1 h), and then labeled only with fluorescein- and rhodamine-conjugated secondary antibodies in order to detect the microinjected rabbit and mouse IgGs directly. (B) Anti-Nup153 monoclonal antibody was microinjected into the cytoplasm of NRK cells, and 1 h post-injection the cells were fixed and permeabilized with either digitonin (0.004%), which leaves the nuclear membranes intact, or Triton X-100 (0.2%), and labeled with rabbit anti-lamin A followed by fluorescein- and rhodamine-conjugated secondary antibodies.

note that a careful analysis of the transport properties of Nup153 in the heterokaryon shuttling assay reveals very slow shuttling (data not shown). It is possible that Nup153 rarely moves sufficiently far out of the body of the pore for a significant number of molecules to accumulate in a heterologous nucleus, or that it rarely dissociates from the cytoplasmic face of the NPC.

Import and export receptors interact with N- and C-terminal regions of Nup153

The identification of a novel type of receptor–nucleoporin interaction that is independent of FG repeats led us to examine the interactions of other transport receptors with Nup153. First, we analyzed the interactions between full-length *in vitro* translated Nup153 and GST–TRN1 or GST–importin β . Both import receptors interact with full-length Nup153, and these interactions are significantly reduced or abolished when the binding assays are performed in the presence of RanQ69L-GTP (Figure 6). The importin β –Nup153 interaction detected here is in agreement with a report that importin β and Nup153 are found in a complex in *Xenopus* oocyte extracts, and this interaction is abolished by the presence of the non-hydrolyzable GTP analog, GMP-PNP (Shah *et al.*, 1998).

Next, we produced purified recombinant GST–Nup153 in three domains (Figure 1), the N-terminal 609 amino acids containing the M9-type NLS and six FG motifs (GST–Nup153-N), the middle portion of the protein comprising amino acids 610–895, which contains four zinc fingers and two FG motifs (GST–Nup153-M), and the C-terminal domain, amino acids 896–1475, which contains 25 of the FG motifs (GST–Nup153-C). These recombinant GST–Nup153 fusions were incubated with *in vitro* translated TRN1, importin β or CRM1. Both import receptors interact with the N- and C-terminal domains of Nup153, and TRN1 also interacts, albeit much more weakly, with

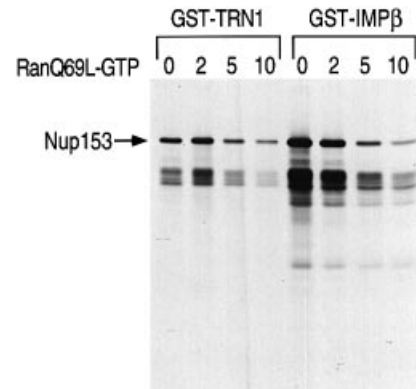


Fig. 6. Full-length Nup153 interacts with TRN1 and importin β *in vitro*, in a RanGTP-regulated manner. Nup153 was translated *in vitro* in the presence of [³⁵S]methionine, and incubated with glutathione–Sepharose beads loaded with 40 pmol of GST–TRN1 or GST–importin β , in the absence or presence of a 2 \times , 5 \times or 10 \times molar excess (over GST–receptor) of RanQ69L-GTP. Bound Nup153 was eluted by boiling the beads in SDS–PAGE sample buffer, and analyzed by SDS–PAGE and autoradiography.

the middle domain (Figure 7). All TRN1–Nup153 interactions are reduced or abolished in the presence of RanQ69L-GTP, while only the importin β –Nup153 N-terminus interaction is sensitive to RanQ69L-GTP. By contrast, the export receptor CRM1 interacts weakly or not at all with Nup153. However, in the presence of RanQ69L-GTP, CRM1 binds to both the N- and C-terminal portions of Nup153 (Figure 7).

We also performed receptor–Nup153 binding assays in which only recombinant, purified proteins were used. Similarly to *in vitro* translated receptors, both recombinant TRN1 and recombinant importin β interact with the N- and C-terminal domains of Nup153 (Figure 8). However, in the all-recombinant assay, TRN1 does not interact

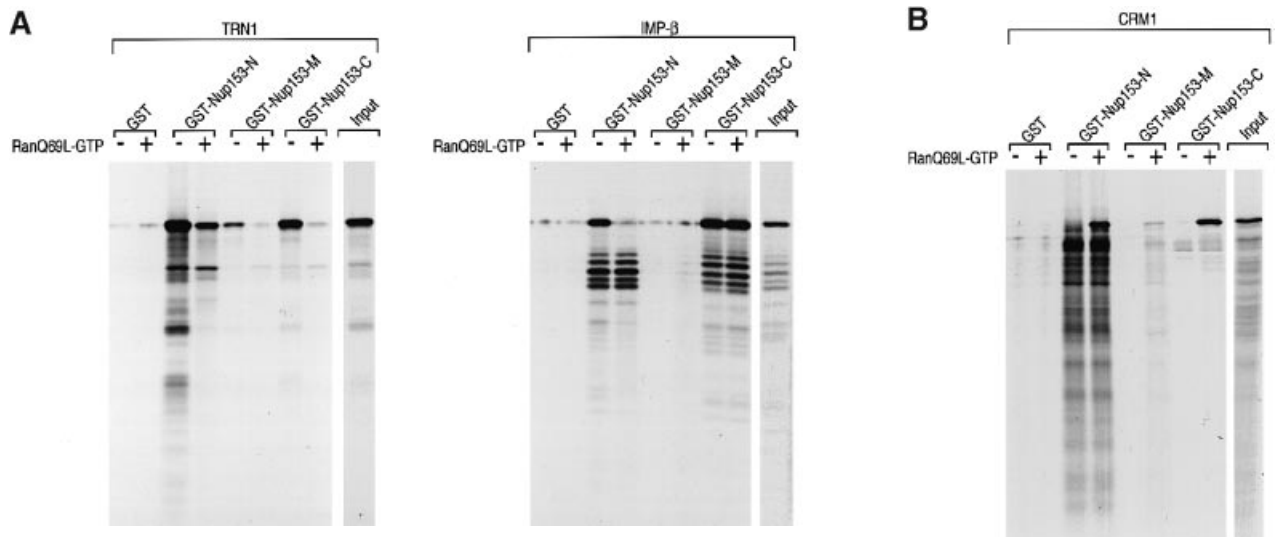


Fig. 7. Import and export receptors interact with several regions of Nup153 in a RanGTP-regulated manner. Import receptors TRN1 and importin β (A), and the export receptor CRM1 (B), were translated *in vitro* in the presence of [35 S]methionine, and incubated with glutathione–Sepharose beads loaded with 5 μ g of GST or GST fused to the N-terminal (N), middle (M) or C-terminal (C) domains of Nup153 (see Figure 1), in the absence or presence of RanQ69L-GTP (6 μ g). Bound receptors were eluted by boiling the beads in SDS–PAGE sample buffer, and analyzed by SDS–PAGE and autoradiography. The input lanes show 1/15 of the protein used in each binding assay.

with the middle portion of Nup153, suggesting that the interaction detected with *in vitro* translated TRN1 may be indirect, mediated by a component of the reticulocyte lysate, or may require another component to stabilize it. Alternatively, the interaction may depend on a post-translational modification of TRN1 that is provided by the reticulocyte lysate.

The response of import receptor–nucleoporin interactions to RanQ69L-GTP is different in the all-recombinant binding assays. First, the TRN1–Nup153 (N) interaction appears stable to RanQ69L-GTP in this assay. Secondly, the importin β –Nup153 (C) interaction is partly destabilized by RanQ69L-GTP in the all-recombinant assay. The explanation for these differences in binding properties between *in vitro* translated and recombinant receptors is not clear, but an interesting possibility is that *in vivo*, these Ran-receptor–Nup153 interactions are subject to additional regulatory factors which may be provided by the reticulocyte lysate of the protein translation system. However, the amount of *in vitro* translated receptor used is much lower than the amount of receptor used in the all-recombinant assays, and the reticulocyte lysate itself contains many transport factors, including receptors and Ran. Therefore, competition from transport factors in the lysate probably contributes to the differences seen in the two binding assays.

The all-recombinant binding assays also revealed direct RanGTP-dependent interactions between CRM1 and the N- and C-terminal domains of Nup153 (Figure 8), indicating that the CRM1–Nup153 interaction is independent of export cargo. We note that, since we have not tested the effect of RanGDP on receptor–Nup153 interactions, we cannot exclude the possibility that RanGDP also influences these interactions.

Nup153 is a Ran-binding protein

In the course of our studies of receptor–Ran–nucleoporin interactions, we detected a novel interaction between RanQ69L-GTP and Nup153. This interaction is restricted

to the middle zinc finger domain of Nup153 (Figure 9). The interaction is direct, since all components in this binding assay are purified recombinant proteins. Ran and Nup153 also interact when the GST tag is on Ran and the His tag is on Nup153 (data not shown). The guanine nucleotide specificity of the Ran–Nup153 interaction was tested. While importin β , as expected, binds specifically to RanGTP (Chi and Adam, 1997; Kutay *et al.*, 1997b), the Nup153 zinc finger domain interacts exclusively with RanGDP (Figure 10). The interaction between Nup153-M and RanQ69L-GTP detected in Figure 9 presumably reflects the presence of RanQ69L-GDP in our RanQ69L-GTP preparation. We conclude that Nup153 is a novel RanGDP-binding protein.

Discussion

Nup153, identified here as a novel cargo for TRN1, plays a role in mRNA export, since overexpression of the protein in mammalian cells results in the accumulation of poly(A)⁺ RNA in the nucleus (Bastos *et al.*, 1996). Shuttling hnRNP proteins have also been implicated to function in mRNA export (Lee *et al.*, 1996; Visa *et al.*, 1996; Nakielny *et al.*, 1997; Pinol-Roma, 1997). Furthermore, microinjection of hnRNP A1 into the nucleus of *Xenopus* oocytes blocks mRNA export (Izaurralde *et al.*, 1997a). Therefore, these import cargos of TRN1 have a common property, they participate in the transport of mRNA.

The identification of an NLS within the N-terminal region of Nup153 is consistent with the previously reported observation that fusion of the N-terminal 609 amino acids of Nup153 to PK directs PK to the nucleoplasm and to the nucleoplasmic face of the NPC (Bastos *et al.*, 1996; Enarson *et al.*, 1998). The M9-type NLS identified here is sufficient to target PK to the nucleoplasm, while other sequences within the N-terminus of Nup153 are necessary for its incorporation into the NPC (Bastos *et al.*, 1996; Enarson *et al.*, 1998). The Nup153 M9-like NLS shows some sequence similarity to the prototypical M9 NLS of

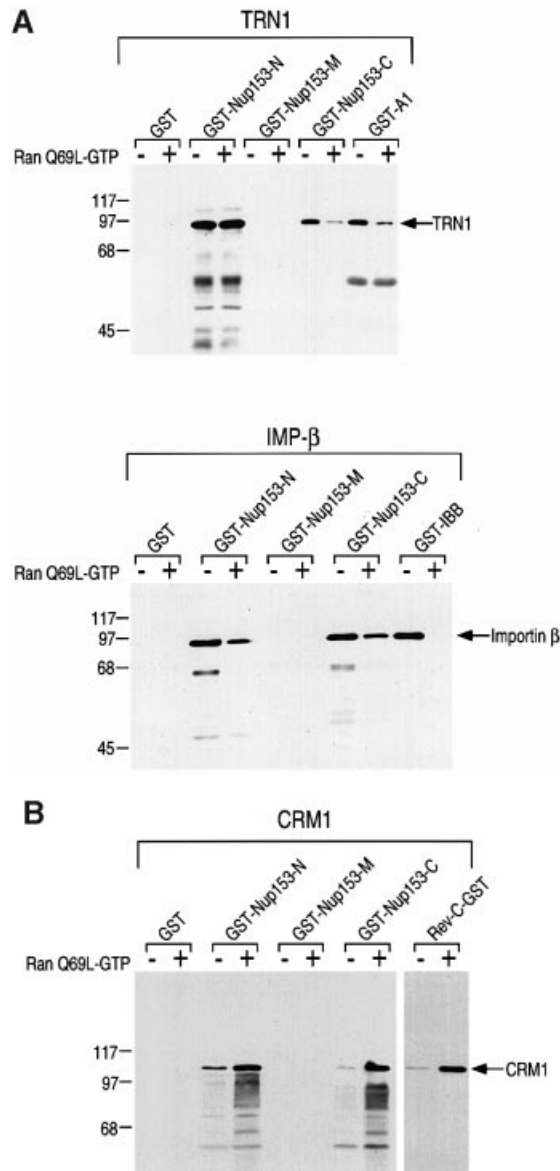


Fig. 8. TRN1, importin β (A) and CRM1 (B) interact directly with the N- and C-terminal domains of Nup153, in a RanGTP-regulated manner. Recombinant TRN1, importin β (~90 pmol of each) or CRM1 (20 pmol) were incubated with glutathione-Sepharose beads loaded with ~70 pmol of GST fusion protein, except GST, GST-Nup153 (M) and Rev-C-GST, for which ~200 pmol were used. Incubations were in the absence or presence of RanQ69L-GTP (180, 360 and 100 pmol for the TRN1, importin β and CRM1 panels, respectively). Bound receptors were eluted by boiling the beads in SDS-PAGE sample buffer, and analyzed by SDS-PAGE and Western blotting with an anti-His tag polyclonal antibody (TRN1 and importin β panels) or with an anti-tetra His monoclonal antibody (CRM1 panel).

hnRNP A1 (Figure 1B), and it appears to bind to the same region of TRN1, because M3 (a larger portion of A1 that spans M9) effectively competes with the Nup153 M9-like NLS for TRN1 binding. In cells treated with inhibitors of RNA pol II, the M9-like NLS of Nup153 relocalizes a reporter protein to the cytoplasm. This suggests that, like A1 M9 (Michael *et al.*, 1995), the M9 domain of Nup153 may function as an NES. However, we have not been able to detect a similar response of either endogenous or transfected full-length Nup153 to RNA pol II inhibition (data not shown). It should be noted, however, that Nup153

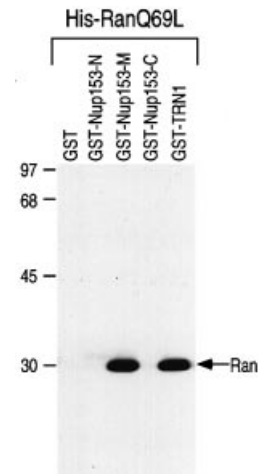


Fig. 9. Nup153 is a Ran-binding protein. The indicated GST fusion proteins (~30 pmol of each) were loaded onto glutathione-Sepharose beads and incubated with His-RanQ69L-GTP (~75 pmol). Bound RanQ69L-GTP was eluted by boiling the beads in SDS-PAGE sample buffer, and analyzed by SDS-PAGE and Western blotting with an anti-Ran monoclonal antibody.

can interact, either directly or indirectly, with importin α (data not shown), suggesting that Nup153 may also be imported along the classical NLS pathway *in vivo*. Classical basic NLSs override transcription-sensitive NLSs, i.e. proteins containing both types of signal do not relocalize to the cytoplasm when pol II is inhibited (Michael *et al.*, 1997). The presence of both types of NLS in Nup153 may explain why it does not show cytoplasmic accumulation upon pol II inhibition. Although inhibitors of pol II did not reveal accumulation of Nup153 in the cytoplasm, the ability of anti-Nup153 antibodies injected into the cytoplasm to move into the nucleus strongly suggests that Nup153 is a mobile protein, capable of moving from its steady-state location in the nucleoplasmic baskets of the NPC, out towards the cytoplasmic face of the NPC.

When we extended our analysis of receptor-nucleoporin interactions to include other import and export receptors, we found that import receptors (TRN1 and importin β) and an export receptor (CRM1) bind well to the N-terminal domain of Nup153 that contains six FG motifs, and to the C-terminal domain that contains 25 FG motifs. That these interactions are likely to be functionally relevant is shown by their response to RanGTP. Import receptor-Nup153 interactions are generally inhibited by RanGTP, while export receptor-Nup153 interactions are dependent on RanGTP. While other import receptor-nucleoporin interactions have been previously demonstrated to be abrogated by RanGTP (Rexach and Blobel, 1995; Delphin *et al.*, 1997; Percipalle *et al.*, 1997), a RanGTP-dependent export receptor-nucleoporin interaction has not been reported. The RanGTP-mediated dissociation of import receptor-nucleoporin interactions at the nucleoplasmic side of the pore has been suggested to be part of the termination step of nuclear import. It is likely that the RanGTP-mediated association of export receptors with nucleoporins at the nucleoplasmic side of the NPC is a very early step in the translocation of cargo-export receptor complexes through the NPC to the cytoplasm. How export cargo-receptor complexes move on from these RanGTP-mediated interactions with Nup153 into the body of the

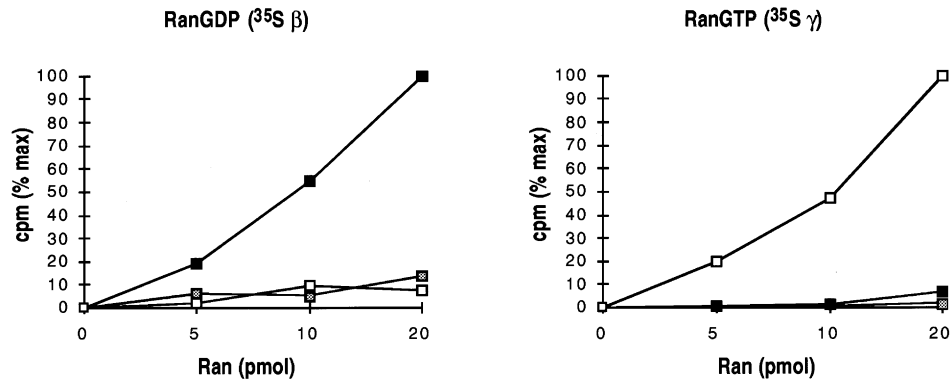


Fig. 10. The zinc finger domain of Nup153 is a novel RanGDP-binding domain. [β - ^{35}S]His-RanGDP or [γ - ^{35}S]His-RanGTP were incubated with glutathione-Sepharose beads loaded with 100 pmol of the indicated GST fusion proteins. After washing, the amount of ^{35}S -label bound to the GST fusion proteins was determined by scintillation counting. Black, GST-Nup153-M; white, GST-importin β ; gray, GST alone.

NPC is an open question. It is possible that binding of the RanGTP-receptor-export cargo complex to Nup153 triggers a dissociation and hand-off of the cargo to more distal components of the pore. However, we suggest, in light of the identification of a shuttling M9 domain in Nup153, and the transient exposure of endogenous Nup153 to anti-Nup153 antibodies injected into the cytoplasm, that this nucleoporin may be a shuttling protein and, therefore, may itself be part of the mobile export complex, playing an important role in the translocation process.

We also report here that Nup153 is a RanGDP-binding protein. The interaction is via the middle domain of Nup153 that contains four zinc fingers and no other recognizable motifs. Although the zinc fingers of Nup153 have been reported to bind DNA *in vitro* (Sukegawa and Blobel, 1993), it is equally likely that this domain constitutes a protein-protein interaction domain (Mackay and Crossley, 1998). Interestingly, other small GTPases have been reported to interact with their effectors via zinc finger domains. For example, rim and rabphilin-3, two putative effectors of the GTPase Rab3 that functions in synaptic vesicle fusion and exocytosis, bind to Rab3 using their zinc fingers (Stahl *et al.*, 1996; Sudhof, 1997; Wang *et al.*, 1997). These zinc finger-GTPase interactions are relevant *in vivo*, since the Rab3-rabphilin interaction was shown to be essential for the proper positioning of rabphilin to exocytotic sites of synapses, and overexpression of the rim zinc finger domain stimulates regulated exocytosis in a Rab3-dependent manner (Sudhof, 1997; Wang *et al.*, 1997). In the case of the Nup153-Ran interaction, the function remains to be investigated.

Two classes of Ran-binding motif have been identified previously. Although both bind specifically to the GTP-bound form of Ran, they differ in their binding sites on Ran, and in their effects on GTP hydrolysis and guanine nucleotide exchange on Ran. The first, exemplified by the Ran-binding domain of RanBP1 (Coutavas *et al.*, 1993; Beddow *et al.*, 1995; Bischoff *et al.*, 1995), is an ~150 amino acid motif found in several other components of the nucleocytoplasmic transport system, for example RanBP2/Nup358 (in vertebrates) (Wu *et al.*, 1995; Yokoyama *et al.*, 1995) and Nup2p (in yeast) (Dingwall *et al.*, 1995; Hartmann and Gorlich, 1995). Ran-binding domains of the RanBP1 class inhibit guanine nucleotide exchange but not GTP hydrolysis (Lounsbury *et al.*, 1994; Bischoff *et al.*, 1995). The second Ran-binding motif is

rather degenerate and is found in the N-termini of transport receptors (Chi and Adam, 1997; Fornerod *et al.*, 1997b; Gorlich *et al.*, 1997; Kutay *et al.*, 1997b). Binding of receptors to RanGTP inhibits both guanine nucleotide exchange and GTP hydrolysis (Floer and Blobel, 1996; Gorlich *et al.*, 1996b). The identification of the zinc finger region as a Ran-binding domain in Nup153 suggests the existence of a third class of Ran-binding motif, which we refer to as the zinc finger Ran-binding domain, or RBZ. It will be of interest to determine the effect of the Nup153-Ran interaction on nucleotide exchange on Ran. The zinc fingers of Nup153 are most closely related to the zinc finger domains of RanBP2/Nup358, and more distantly to a number of other proteins, including RNP motif proteins (Figure 11; McMorro *et al.*, 1994; Yokoyama *et al.*, 1995). This motif defines a novel class of zinc finger that is evolutionarily well conserved (Figure 11). Whether any of these fingers, in particular those of RanBP2/Nup358, can interact with Ran will need to be determined.

The parallels between RanBP2/Nup358 and Nup153 are intriguing. First, they are both localized in distal structures that emanate from the NPC, but RanBP2/Nup358 is a component of the cytoplasmic fibrils (Wilken *et al.*, 1995; Wu *et al.*, 1995; Yokoyama *et al.*, 1995), while Nup153 is in the nucleoplasmic basket structure (Sukegawa and Blobel, 1993; Pante *et al.*, 1994). Secondly, both bind transport receptors in a RanGTP-regulated manner. RanGTP facilitates import receptor interactions with RanBP2/Nup358 (Delphin *et al.*, 1997) and export receptor interactions with Nup153 (this study), and dissociates import receptor-Nup153 interactions (Shah *et al.*, 1998; this study). Thirdly, at the level of primary structure, these two nucleoporins appear to be related to each other. Specifically, the zinc finger domain of RanBP2/Nup358, together with a region of ~100 residues C-terminal to this domain, are more similar to the equivalent regions of Nup153 than to any other protein in the database (Yokoyama *et al.*, 1995). Finally, RanBP2/Nup358 and Nup153 are currently the only known Ran-binding proteins within the vertebrate NPC. Based on these properties, it is very likely that RanBP2/Nup358 and Nup153 have parallel functions at the cytoplasmic and nucleoplasmic termini of the NPC, respectively. Their localizations position them for primary roles in the initiation of transport (RanBP2/Nup358 for import, Nup153 for export) and in

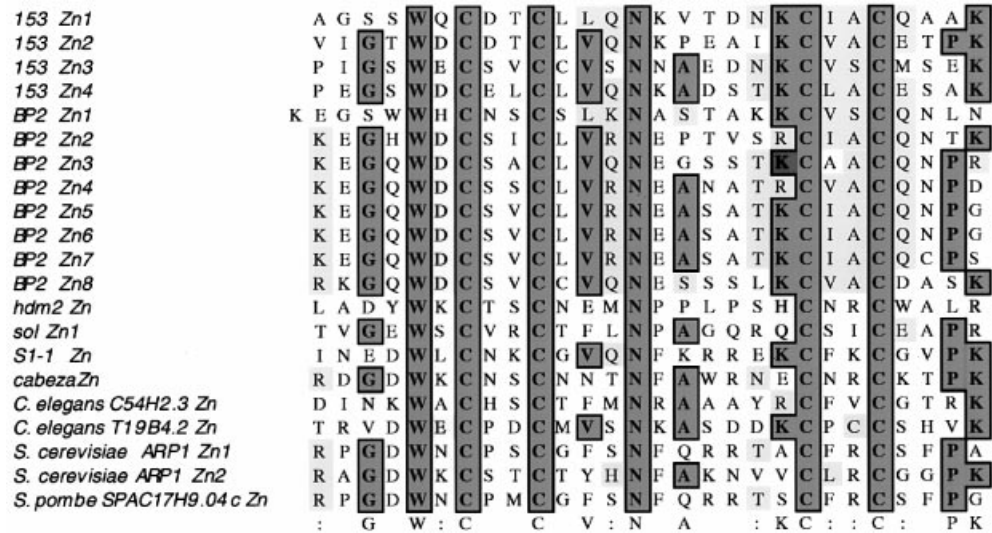


Fig. 11. The Nup153 zinc finger Ran-binding domain (RBZ) is very similar to the zinc fingers of several other proteins. The alignment was done using the ClustalW program. Identical residues are in dark shading and related residues are in pale shading. Sequences are from human proteins unless noted otherwise. The zinc fingers of the following proteins are shown: Nup153 (153; McMorrow *et al.*, 1994), RanBP2/Nup358 (BP2; Yokoyama *et al.*, 1995), oncoprotein hdm2 (Oliner *et al.*, 1992), small optic lobes (sol, one of the five fingers in sol is shown; Kamei *et al.*, 1998), rat RNP motif protein S1-1 (Inoue *et al.*, 1996), *Drosophila* RNP motif protein cabeza (Stolow and Haynes, 1995) and *Saccharomyces cerevisiae* ARP1 protein (Wehner *et al.*, 1993). The *Caenorhabditis elegans* C54H2.3 gene product shows similarity to human YY1-associated factor 2 and to yeast Nup2p, and the T19B4.2 gene product is weakly similar to nucleoporins. The *C. elegans* genome contains four additional sequences that encode this type of finger (not shown). The *Schizosaccharomyces pombe* SPAC17H9.04c gene product is similar to *S. cerevisiae* ARP1. A consensus sequence for this type of zinc finger is shown beneath the alignment.

the termination of transport (RanBP2/Nup358 for export, Nup153 for import). Ran–nucleoporin interactions may coordinate and regulate these critical stages of translocation.

Materials and methods

Phage display

A HeLa cDNA library was constructed from HeLa mRNA and the T7Select 1-1b vector (Novagen T7 Select System Manual). The construction of the library and the selection protocols will be described in detail elsewhere. Briefly, this produces fusions of HeLa proteins and the coat protein of bacteriophage T7 which are expressed on the surface of the phage. The random primed library contained 2×10^6 independent clones, and >95% of the phage contained inserts, ranging in size from 0.2 to 1.2 kb. To screen the library for TRN1-interacting proteins, the library (20 μ l aliquot) was first pre-cleared by incubating with GST (20 μ g) immobilized on glutathione–Sepharose beads (50 μ l slurry) in 700 μ l of transport buffer [TB; 20 mM HEPES pH 7.3, 110 mM potassium acetate, 5 mM sodium acetate, 2 mM magnesium acetate, 0.5 mM EGTA, 1 mM dithiothreitol (DTT) and 1 μ g/ml each of leupeptin, aprotinin and pepstatin]. This mix was incubated at 4°C for 1 h, with end-over-end inverting, then the supernatant was loaded onto GST–TRN1 bait (10 μ g) immobilized on glutathione–Sepharose beads. The bait and phage were incubated as above for 1 h, then the beads were washed five times with TB plus 0.1 M potassium chloride. Phage that had been captured by the bait were eluted with glutathione elution buffer (10 mM glutathione, 50 mM Tris–HCl pH 8). Two 15 min room temperature elutions of 50 μ l each were pooled. To amplify the selected phage, a 50 μ l aliquot of the elution was incubated (37°C shaker) with *Escherichia coli* strain BLT5403 (5 ml of OD ~0.6). Typically, lysis of the bacteria was complete within ~3 h. Bacterial debris was pelleted, and 200 μ l of the phage-containing supernatant was used for cycle 2 of the screening. Four cycles were performed, and the phage were pre-cleared on glutathione–Sepharose beads at every cycle. After the final cycle, the phage were used to lyse plated *E. coli*. Single plaque plugs were incubated in 100 μ l of SM (1 h at room temperature), and a 10 μ l aliquot of the eluted phage was used for PCR amplification of the phage insert (primers T7Select UP and T7Select DOWN, Novagen). The PCR products were purified (QIAquick PCR purification kit, Qiagen) and sequenced.

Plasmid construction

The eukaryotic expression plasmid encoding myc-PK–Nup153[235–300] was prepared by *Pfu* PCR amplification of this region of Nup153 with primers that introduce a 5' *Xho*I site, and a 3' stop codon and *Xba*I site. This fragment was inserted into pcDNA3-myc-PK (Nakielny and Dreyfuss, 1996) digested with *Xho*I and *Xba*I. The prokaryotic expression plasmid encoding GST–Nup153[230–305]–His was made by *Pfu* PCR amplification of this region of Nup153 with primers that introduce a 5' *Bam*HI site and a 3' *Hind*III site. This was inserted into *Bam*HI–*Hind*III-digested pETGST II vector. The vector was made by inserting *Pfu* PCR-amplified GST cDNA containing a 5' *Nco*I site and a 3' *Bam*HI site into *Nco*I–*Bam*HI-digested pET28a (Novagen). The prokaryotic expression plasmids encoding GST–Nup153-N, M and C were produced by *Pfu* PCR amplification of Nup153 cDNA corresponding to amino acids 2–609, 610–895 and 896–1475, respectively. For GST–Nup153-N and GST–Nup153-M, the primers introduced a 5' *Bam*HI site, and a 3' stop codon and *Sal*I site, and for GST–Nup153-C, the primers introduced a 5' *Sal*I site and a 3' *Not*I site (the stop codon was amplified from the Nup153 cDNA). The three fragments were inserted into appropriately digested pGEX 5X-3 (Pharmacia). The prokaryotic expression plasmid encoding MBP was made by digesting pMAL-c2 (New England Biolabs), which encodes an MBP–lacZ fusion protein, with *Xba*I. The 3' recessed ends were filled in with Klenow, and the vector was re-ligated. This generates an in-frame stop codon between MBP and lacZ. The plasmid encoding MBP–IBB was made by *Pfu* PCR amplification of the region corresponding to amino acids 2–65 of human Rch1 cDNA, to generate a 5' *Bam*HI site, and a 3' stop codon and *Hind*III site. The fragment was inserted into *Bam*HI–*Hind*III-digested pMAL-c2. To make the plasmid encoding His-A1, full-length A1 was isolated as an *Eco*RI–*Xho*I fragment from pcDNA3-myc-A1 (Nakielny and Dreyfuss, 1996), and inserted into *Eco*RI–*Xho*I-digested pET28b (Novagen). The plasmid encoding His-Rev-C-terminus–GST (Rev-C–GST) was made by PCR amplification of a Rev cDNA corresponding to amino acids 56–116, using primers that introduce a 5' *Eco*RI site and a 3' *Hind*III site. This was inserted into *Eco*RI–*Hind*III-digested vector pETGST I. The vector was made by inserting a *Pfu* PCR-amplified GST fragment containing a 5' *Hind*III site and a 3' *Xho*I site into *Hind*III–*Xho*I-digested pET28a (Novagen). Construction of the following plasmids has been described previously: pcDNA3-myc-PK, pcDNA3-myc-PK-M9 (Siomi and Dreyfuss, 1995), GST–TRN1 and GST–importin β (Nakielny and Dreyfuss, 1997), GST–A1 (Nakielny and Dreyfuss, 1996), GST–M9, pET28C–TRN1 and pCR–importin β (Pollard *et al.*, 1996), GST–IBB

(Michael *et al.*, 1997), pcDNA3-myc-A1 (Nakielny and Dreyfuss, 1996) and zz-M3 (Siomi *et al.*, 1997).

Recombinant protein expression and purification

All proteins (unless noted) were overexpressed in BL21 (DE3) cells, and the purified proteins were quick-frozen in liquid nitrogen and stored at -80°C .

GST-TRN1 and GST-importin β were induced with 0.2 mM isopropyl- β -D-thiogalactopyranoside (IPTG) overnight at 30°C . The proteins were purified according to the manufacturer's instructions (Pharmacia), and all buffers contained 2 $\mu\text{g}/\text{ml}$ each of leupeptin, pepstatin and aprotinin, and 1 mM DTT. The purified proteins were dialyzed into TB (see phage display) with 10% glycerol. GST-A1 was induced with 0.1 mM IPTG for 3 h at 37°C . The protein was purified according to the manufacturer's instructions (Pharmacia), and dialyzed into phosphate-buffered saline (PBS). GST-Nup153-N, M and C were induced with 0.2 mM IPTG (4 h at 37°C), purified according to the manufacturer's instructions (Pharmacia) and dialyzed into PBS with 10% glycerol. GST-Nup153-N, M and C recombinant proteins suffered extensive proteolysis, despite the inclusion of 10 $\mu\text{g}/\text{ml}$ each of leupeptin, pepstatin, aprotinin and 1 mM EDTA in all buffers. GST-Nup153[230–305]-His was induced with 1 mM IPTG (4 h at 37°C), and the protein was purified on nickel resin according to the manufacturer's instructions (Novagen) and dialyzed into TB. His-TRN1 was induced with 1 mM IPTG for 4 h at 17°C , purified according to the manufacturer's instructions (Novagen) and dialyzed into PBS with 10% glycerol. All buffers contained 1 $\mu\text{g}/\text{ml}$ each of leupeptin, pepstatin, aprotinin and 0.1 mM TCEP (Pierce). His-Stag-importin β -myc was induced for 3 h at 30°C with 1 mM IPTG. The pelleted bacteria were resuspended in bind buffer (50 mM Tris-HCl pH 7.5, 0.2 M NaCl, 20 mM imidazole, 1 mM MgCl_2) and incubated on ice for 1 h with lysozyme (1 mg/ml final). After centrifugation, the supernatant was loaded onto nickel resin equilibrated in bind buffer, then the resin was washed with bind buffer plus 20 mM imidazole, and the protein was eluted with bind buffer plus 380 mM imidazole and dialyzed into TB. All buffers contained 1 $\mu\text{g}/\text{ml}$ each of leupeptin, pepstatin, aprotinin and 0.1 mM TCEP (Pierce). His-A1 was induced for 2.5 h at 37°C with 1 mM IPTG, purified on nickel resin according to the manufacturer's instructions (Novagen) and dialyzed against TB with 5% glycerol. Precipitated material was evident after elution from the resin and after dialysis, but a good yield of soluble His-A1 was obtained. His-RanQ69L-GTP, His-RanGDP and His-RanGTP were prepared by induction in M15[pREP4] cells with 1 mM IPTG for 3 h at 37°C . All buffers contained 0.1 mM GDP or GTP, 1 $\mu\text{g}/\text{ml}$ each of leupeptin, pepstatin, aprotinin and 0.1 mM TCEP (Pierce). Pelleted cells were resuspended in buffer A (50 mM HEPES pH 7.4, 100 mM NaCl, 10 mM magnesium acetate), sonicated, centrifuged, and the supernatant was loaded onto nickel resin equilibrated in buffer A. After washing the resin with buffer A plus 400 mM NaCl and 40 mM imidazole, the Ran proteins were eluted with buffer A plus 400 mM NaCl and 80 mM imidazole, and dialyzed into TB with 10% glycerol. MBP and MBP-IBB were induced with 0.3 mM IPTG for 4 h at 37°C , purified on amylose resin according to the manufacturer's instructions (NEB) and dialyzed into TB. His-Rev-C-GST was induced with 1 mM IPTG for 5 h at 37°C , and purified on nickel resin according to the instructions (Novagen). Protein eluted from the nickel resin was pooled and diluted 1:5 with PBS, purified on glutathione resin as instructed (Pharmacia) and dialyzed into PBS with 10% glycerol. GST-M9 and GST-IBB were expressed and purified as described previously (Pollard *et al.*, 1996; Michael *et al.*, 1997), and recombinant CRM1-His was generously provided by Maarten Fornerod.

In vitro transcription-translation (TNT)

Coupled *in vitro* transcription/translation was done according to the manufacturer's instructions (Promega), in the presence of ^{35}S -labeled methionine. Protein products of the reaction were checked by SDS-PAGE and autoradiography before use in protein-protein interaction assays. The efficiency of binding was routinely 5–15% of the input.

Protein-protein interaction assays

All incubations were done at 4°C , and all buffers contain 1 $\mu\text{g}/\text{ml}$ each of leupeptin, pepstatin and aprotinin, and 1 mM DTT. GST fusion proteins (the amounts are indicated in the figure legends) were loaded onto 40 μl of glutathione-Sepharose beads for 1 h in 500 μl of TB (see phage display) or PBS. After washing with $2 \times 1 \text{ ml}$ of TB, the loaded beads were incubated for 1 h in 1 ml of 50 mM Tris (pH 7.5), 400 mM NaCl, 2.5 mM magnesium acetate plus 0.05% digitonin (Figure 2A) or in 1 ml of TB plus 0.05% digitonin (all other figures), together with

15 μl of ^{35}S -labeled protein from the TNT reaction (above), or with recombinant purified His-tagged proteins. Ran proteins were included in this incubation as indicated in the figure legends. The beads were then washed with $5 \times 1 \text{ ml}$ of 50 mM Tris (pH 7.5), 400 mM NaCl, 2.5 mM magnesium acetate plus 0.05% digitonin (Figure 2A) or with $5 \times 1 \text{ ml}$ of TB plus 0.05% digitonin (all other figures), boiled in SDS-PAGE sample buffer and analyzed by SDS-PAGE and autoradiography or Western blotting.

In vitro protein import assay

This was performed as described (Nakielny and Dreyfuss, 1997), except that each assay contained 45% (v/v) reticulocyte lysate as a source of transport factors, together with GST-M9 (1.5 μM), GST-IBB (1.5 μM) or GST-Nup153[230–305] (1 μM). The competitors were used at a concentration of 10 μM .

Microinjections

The SA1 monoclonal IgG specific for Nup153 was purified from spent tissue culture medium by chromatography on protein G-Sepharose. Antibodies, both SA1 IgG and non-immune rabbit IgG, were used at a final concentration of 1 mg/ml in PBS and microinjected into the nucleus or cytoplasm of NRK cells employing an Eppendorf Automated Microinjection System. To inhibit protein synthesis, the cells were incubated in the presence of 20 $\mu\text{g}/\text{ml}$ cycloheximide starting 1 h prior to the injections and continuing for the duration of the post-injection incubations.

Cell culture and transfections

These procedures were as detailed in Nakielny and Dreyfuss (1996).

Immunofluorescence, confocal microscopy and Western blotting

These were performed as described previously (Nakielny and Dreyfuss, 1997).

Loading of Ran protein with radiolabeled guanine nucleotides

His-RanGDP or His-RanGTP (25 μg) were incubated on ice in the presence of 20 mM HEPES (pH 7.4), 50 mM potassium acetate, 2.5 mM sodium acetate, 1 mM magnesium acetate, 0.25 mM EGTA, 2 mM DTT, 5% glycerol, 50 μM cold GDP or GTP, 0.3 μM [β - ^{35}S]GDP or [γ - ^{35}S]GTP (50 μCi), together with 10 mM EDTA. After 45 min, magnesium acetate was added to 20 mM final, and the incubation was continued for a further 30 min on ice. [β - ^{35}S]His-RanGDP and [γ - ^{35}S]His-RanGTP were quick frozen in liquid nitrogen and stored at -80°C .

Antibodies

The anti-myc monoclonal antibody 9E10 was used at 1:3000, the anti-GST monoclonal and anti-His polyclonal (both Santa Cruz Biotechnology) were used at 1:100 and 1:1000, respectively, the anti-Ran monoclonal antibody (Transduction Laboratories) was used at 1:5000, and the anti-tetra His monoclonal antibody (Qiagen) was resuspended to 0.2 mg/ml and used at 1:2000. The affinity-purified anti-peptide antibody against lamin A and the SA1 monoclonal IgG specific for Nup153 are described elsewhere (Powell and Burke, 1990; K.Bodoor, S.A.Shaikh, W.H.Raharjo, R.Bastos, M.J.Lohka and B.Burke, submitted).

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References

- Adam, S.A., Marr, R.S. and Gerace, L. (1990) Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J. Cell Biol.*, **111**, 807–816.
- Aitchison, J.D., Blobel, G. and Rout, M.P. (1996) Kap104p: a karyopherin involved in the nuclear transport of messenger RNA binding proteins. *Science*, **274**, 624–627.
- Arts, G.-J., Fornerod, M. and Mattaj, J.W. (1998) Identification of a nuclear export receptor for tRNA. *Curr. Biol.*, **8**, 305–314.
- 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.
- Bastos, R., de Pouplana, L.R., Enarson, M., Bodoor, K. and Burke, B. (1997) Nup84, a novel nucleoporin that is associated with CAN/Nup214 on the cytoplasmic face of the nuclear pore complex. *J. Cell Biol.*, **137**, 989–1000.
- Beddow, A.L., Richards, S.A., Orem, N.R. and Macara, I.G. (1995) The Ran/TC4 GTPase-binding domain: identification by expression cloning and characterization of a conserved sequence motif. *Proc. Natl Acad. Sci. USA*, **92**, 3328–3332.
- Bertolotti, A., Lutz, Y., Heard, D.J., Chambon, P. and Tora, L. (1996) hTAF_{II}68, a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS, is associated with both TFIID and RNA polymerase II. *EMBO J.*, **15**, 5022–5031.
- Bischoff, F.R. and Gorlich, D. (1997) RanBP1 is crucial for the release of RanGTP from importin β -related nuclear transport factors. *FEBS Lett.*, **419**, 249–254.
- Bischoff, F.R., Krebber, H., Smirnova, E., Dong, W. and Ponstingl, H. (1995) Co-activation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. *EMBO J.*, **14**, 705–715.
- Boche, I. and Fanning, E. (1997) Nucleocytoplasmic recycling of the nuclear localization signal receptor alpha subunit *in vivo* is dependent on a nuclear export signal, energy and RCC1. *J. Cell Biol.*, **139**, 313–325.
- Bonifaci, N., Moroianu, J., Radu, A. and Blobel, G. (1997) Karyopherin β 2 mediates nuclear import of a mRNA binding protein. *Proc. Natl Acad. Sci. USA*, **94**, 5055–5060.
- Chi, N.C. and Adam, S.A. (1997) Functional domains in nuclear import factor p97 for binding the nuclear localization sequence receptor and the nuclear pore. *Mol. Biol. Cell*, **8**, 945–956.
- Cole, C.N. and Saavedra, C. (1997) Regulation of the export of RNA from the nucleus. *Semin. Cell Dev. Biol.*, **8**, 71–78.
- Cordes, V.C., Reidenbach, S., Rackwitz, H.-R. and Franke, W.W. (1997) Identification of protein p270/Tpr as a constitutive component of the nuclear pore complex-attached intranuclear filaments. *J. Cell Biol.*, **136**, 515–529.
- Coutavas, E., Ren, M., Oppenheim, J.D., D'Eustachio, P. and Rush, M.G. (1993) Characterization of proteins that interact with the cell-cycle regulatory protein Ran/TC4. *Nature*, **366**, 585–587.
- Dahlberg, J.E. and Lund, E. (1998) Functions of the GTPase Ran in RNA export from the nucleus. *Curr. Opin. Cell Biol.*, **10**, 400–408.
- Davis, L.I. (1995) The nuclear pore complex. *Annu. Rev. Biochem.*, **64**, 865–896.
- Delphin, C., Guan, T., Melchior, F. and Gerace, L. (1997) RanGTP targets p97 to RanBP2, a filamentous protein localized at the cytoplasmic periphery of the nuclear pore complex. *Mol. Biol. Cell*, **8**, 2379–2390.
- Dingwall, C., Kandels-Lewis, S. and Seraphin, B. (1995) A family of Ran binding proteins that includes nucleoporins. *Proc. Natl Acad. Sci. USA*, **92**, 7525–7529.
- Doye, V. and Hurt, E. (1997) From nucleoporins to nuclear pore complexes. *Curr. Opin. Cell Biol.*, **9**, 401–411.
- Enarson, P., Enarson, M., Bastos, R. and Burke, B. (1998) Amino-terminal sequences that direct nucleoporin Nup153 to the inner surface of the nuclear envelope. *Chromosoma*, **107**, 228–236.
- Floer, M. and Blobel, G. (1996) The nuclear transport factor karyopherin β binds stoichiometrically to Ran-GTP and inhibits the Ran GTPase activating protein. *J. Biol. Chem.*, **271**, 5313–5316.
- Fornerod, M., Ohno, M., Yoshida, M. and Mattaj, J.W. (1997a) CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell*, **90**, 1051–1060.
- Fornerod, M., van Deursen, J., van Baal, S., Reynolds, A., Davis, D., Murti, K.G., Fransen, J. and Grosveld, G. (1997b) The human homologue of yeast CRM1 is in a dynamic subcomplex with CAN/Nup214 and a novel nuclear pore component Nup88. *EMBO J.*, **16**, 807–816.
- Fridell, R.A., Truant, R., Thorne, L., Benson, R.E. and Cullen, B.R. (1997) Nuclear import of hnRNP A1 is mediated by a novel cellular cofactor related to karyopherin- β . *J. Cell Sci.*, **110**, 1325–1331.
- Fukuda, M., Asano, S., Nakamura, T., Adachi, M., Yoshida, M., Yanagida, M. and Nishida, E. (1997) CRM1 is responsible for intracellular transport mediated by the nuclear export signal. *Nature*, **390**, 308–311.
- Gorlich, D. (1998) Transport into and out of the cell nucleus. *EMBO J.*, **17**, 2721–2727.
- Gorlich, D., Henklein, P., Laskey, R.A. and Hartmann, E. (1996a) A 41 amino acid motif in importin- α confers binding to importin- β and hence transit into the nucleus. *EMBO J.*, **15**, 1810–1817.
- Gorlich, D., Pante, N., Kutay, U., Aebi, U. and Bischoff, F.R. (1996b) Identification of different roles for RanGDP and RanGTP in nuclear protein import. *EMBO J.*, **15**, 5584–5594.
- Gorlich, D., Dabrowski, M., Bischoff, F.R., Kutay, U., Bork, P., Hartmann, E., Prehn, S. and Izaurralde, E. (1997) A novel class of RanGTP binding proteins. *J. Cell Biol.*, **138**, 65–80.
- Grandi, P., Dang, T., Pane, N., Shevchenko, A., Mann, M., Forbes, D. and Hurt, E. (1997) Nup93, a vertebrate homologue of yeast Nic96p, forms a complex with a novel 205-kDa protein and is required for correct nuclear pore assembly. *Mol. Biol. Cell*, **8**, 2017–2038.
- Guan, T., Muller, S., Klier, G., Pante, N., Blevitt, J.M., Haner, M., Paschal, B., Aebi, U. and Gerace, L. (1995) Structural analysis of the p62 complex, an assembly of O-linked glycoproteins that localizes near the central gated channel of the nuclear pore complex. *Mol. Biol. Cell*, **6**, 1591–1603.
- Hartmann, E. and Gorlich, D. (1995) A Ran-binding motif in nuclear pore proteins. *Trends Cell Biol.*, **5**, 192–193.
- Herold, A., Truant, R., Wiegand, H. and Cullen, B.R. (1998) Determination of the functional domain organization of the importin α nuclear import factor. *J. Cell Biol.*, **143**, 309–318.
- Hu, T., Guan, T. and Gerace, L. (1996) Molecular and functional characterization of the p62 complex, an assembly of nuclear pore complex glycoproteins. *J. Cell Biol.*, **134**, 589–601.
- Inoue, A., Takahashi, K.P., Kimura, M., Watanabe, T. and Morisawa, S. (1996) Molecular cloning of an RNA binding protein, S1-1. *Nucleic Acids Res.*, **24**, 2990–2997.
- Iovine, M.K., Watkins, J.L. and Wente, S.R. (1995) The GLFG repetitive region of the nucleoporin Nup116p interacts with Kap95p, an essential yeast nuclear import factor. *J. Cell Biol.*, **131**, 1699–1713.
- Izaurralde, E. and Adam, S. (1998) Transport of macromolecules between the nucleus and the cytoplasm. *RNA*, **4**, 351–364.
- Izaurralde, E., Jarmolowski, A., Beisel, C., Mattaj, J.W., Dreyfuss, G. and Fischer, U. (1997a) A role for the M9 transport signal of hnRNP A1 in mRNA nuclear export. *J. Cell Biol.*, **137**, 27–35.
- Izaurralde, E., Kutay, U., von Kobbe, C., Mattaj, J.W. and Gorlich, D. (1997b) 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.
- Jakel, S. and Gorlich, D. (1998) Importin β , transportin, RanBP5 and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells. *EMBO J.*, **17**, 4491–4502.
- Kaffman, A., Miller Rank, N., O'Neill, E.M., Huang, L.S. and O'Shea, E.K. (1998) The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus. *Nature*, **396**, 482–486.
- Kamei, M., Webb, G.C., Young, I.G. and Campbell, H.D. (1998) *SOLH*, a human homologue of the *Drosophila melanogaster small optic lobes* gene is a member of the calpain and zinc-finger gene families and maps to human chromosome 16p13.3 near *CATM* (cataract with microphthalmia). *Genomics*, **51**, 197–206.
- Klebe, C., Bischoff, F.R., Ponstingl, H. and Wittinghofer, A. (1995) Interaction of the nuclear GTP-binding protein Ran with its regulatory proteins RCC1 and RanGAP1. *Biochemistry*, **34**, 639–647.
- 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.
- Kraemer, D., Wozniak, R.W., Blobel, G. and Radu, A. (1994) The human CAN protein, a putative oncogene product associated with myeloid leukemogenesis, is a nuclear pore complex protein that faces the cytoplasm. *Proc. Natl Acad. Sci. USA*, **91**, 1519–1523.
- Kraemer, D.M., Strambio-de-Castillia, C., Blobel, G. and Rout, M.P. (1995) The essential yeast nucleoporin NUP159 is located on the cytoplasmic

- side of the nuclear pore complex and serves in karyopherin-mediated binding of transport substrate. *J. Biol. Chem.*, **270**, 19017–19021.
- Kutay,U., Bischoff,F.R., Kostka,S., Kraft,R. and Gorlich,D. (1997a) Export of importin α from the nucleus is mediated by a specific nuclear transport factor. *Cell*, **90**, 1061–1071.
- Kutay,U., Izaurralde,E., Bischoff,F.R., Mattaj,I.W. and Gorlich,D. (1997b) Dominant-negative mutants of importin- β block multiple pathways of import and export through the nuclear pore complex. *EMBO J.*, **16**, 1153–1163.
- Kutay,U., Lipowsky,G., Izaurralde,E., Bischoff,F.R., Schwarzmaier,P., Hartmann,E. and Gorlich,D. (1998) Identification of a tRNA-specific nuclear export receptor. *Mol. Cell*, **1**, 359–369.
- Lee,M.S., Henry,M. and Silver,P.A. (1996) A protein that shuttles between the nucleus and the cytoplasm is an important mediator of RNA export. *Genes Dev.*, **10**, 1233–1246.
- Lounsbury,K.M., Beddow,A.L. and Macara,I.G. (1994) A family of proteins that stabilize the Ran/TC4 GTPase in its GTP-bound conformation. *J. Biol. Chem.*, **269**, 11285–11290.
- Mackay,J. and Crossley,M. (1998) Zinc fingers are sticking together. *Trends Biochem. Sci.*, **23**, 1–4.
- Mahajan,R., Delphin,C., Guan,T., Gerace,L. and Melchior,F. (1997) A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell*, **88**, 97–107.
- Mattaj,I.W. and Englmeier,L. (1998) Nucleocytoplasmic transport: the soluble phase. *Annu. Rev. Biochem.*, **67**, 265–306.
- Matunis,M.J., Coutavas,E. and Blobel,G. (1996) A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J. Cell Biol.*, **135**, 1457–1470.
- McMorrow,I., Bastos,R., Horton,H. and Burke,B. (1994) Sequence analysis of a cDNA encoding a human nuclear pore complex protein, hnp153. *Biochim. Biophys. Acta*, **1217**, 219–223.
- Melchior,F. and Gerace,L. (1998) Two-way trafficking with Ran. *Trends Cell Biol.*, **8**, 175–179.
- Michael,W.M., Choi,M. and Dreyfuss,G. (1995) A nuclear export signal in hnRNP A1: a signal-mediated, temperature-dependent nuclear protein export pathway. *Cell*, **83**, 415–422.
- Michael,W.M., Eder,P.S. and Dreyfuss,G. (1997) The K nuclear shuttling domain: a novel signal for nuclear import and nuclear export in the hnRNP K protein. *EMBO J.*, **16**, 3587–3598.
- Morohoshi,F., Arai,K., Takahashi,E.-i., Tanigami,A. and Ohki,M. (1996) Cloning and mapping of a human *RBP56* gene encoding a putative RNA binding protein similar to FUS/TLS and EWS proteins. *Genomics*, **38**, 51–57.
- Nakielny,S. and Dreyfuss,G. (1996) The hnRNP C proteins contain a nuclear retention sequence that can override nuclear export signals. *J. Cell Biol.*, **134**, 1365–1373.
- Nakielny,S. and Dreyfuss,G. (1997) Import and export of the nuclear protein import receptor transportin by a mechanism independent of GTP hydrolysis. *Curr. Biol.*, **8**, 89–95.
- Nakielny,S., Siomi,M.C., Siomi,H., Michael,W.M., Pollard,V. and Dreyfuss,G. (1996) Transportin: nuclear transport receptor of a novel nuclear protein import pathway. *Exp. Cell Res.*, **229**, 261–266.
- Nakielny,S., Fischer,U., Michael,W.M. and Dreyfuss,G. (1997) RNA transport. *Annu. Rev. Neurosci.*, **20**, 269–301.
- Neville,M., Stutz,F., Lee,L., Davis,L.I. and Rosbash,M. (1997) The importin- β family member Crm1p bridges the interaction between Rev and the nuclear pore complex during nuclear export. *Curr. Biol.*, **7**, 767–775.
- Nigg,E.A. (1997) Nucleocytoplasmic transport: signals, mechanisms and regulation. *Nature*, **386**, 779–787.
- Oliner,J.D., Kinzler,K.W., Meltzer,P.S., George,D.L. and Vogelstein,B. (1992) Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature*, **358**, 80–83.
- Ossareh-Nazari,B., Bachelier,F. and Dargemont,C. (1997) Evidence for a role of CRM1 in signal-mediated nuclear protein export. *Science*, **278**, 141–144.
- Pante,N., Bastos,R., McMorrow,I., Burke,B. and Aebi,U. (1994) Interactions and three-dimensional localization of a group of nuclear pore complex proteins. *J. Cell Biol.*, **126**, 603–617.
- Percipalle,P., Clarkson,W.D., Kent,H.M., Rhodes,D. and Stewart,M. (1997) Molecular interactions between the importin α/β heterodimer and proteins involved in vertebrate nuclear protein import. *J. Mol. Biol.*, **226**, 722–732.
- Pinol-Roma,S. (1997) hnRNP proteins and the nuclear export of mRNA. *Semin. Cell Dev. Biol.*, **8**, 57–63.
- Pinol-Roma,S. and Dreyfuss,G. (1992) Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. *Nature*, **355**, 730–732.
- 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*, **86**, 985–994.
- Powell,L. and Burke,B. (1990) Internuclear exchange of an inner nuclear membrane protein (p55) in heterokaryons: *in vivo* evidence for the association of p55 with the nuclear lamina. *J. Cell Biol.*, **111**, 2225–2234.
- Radu,A., Moore,M.S. and Blobel,G. (1995) 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.
- Richards,S.A., Carey,K.L. and Macara,I.G. (1997) Requirement of guanosine triphosphate-bound Ran for signal-mediated nuclear protein export. *Science*, **276**, 1842–1844.
- Ribbeck,K., Lipowsky,G., Kent,H.M., Stewart,M. and Gorlich,D. (1998) NTF2 mediates nuclear import of Ran. *EMBO J.*, **17**, 6587–6598.
- Rout,M.P. and Wente,S.R. (1994) Pores for thought: nuclear pore complex proteins. *Trends Cell Biol.*, **4**, 357–365.
- Rush,M.G., Drivas,G. and D'Eustachio,P. (1996) The small nuclear GTPase Ran: how much does it run? *BioEssays*, **18**, 103–112.
- 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**, 31–49.
- Siomi,H. and Dreyfuss,G. (1995) A nuclear localization domain in the hnRNP A1 protein. *J. Cell Biol.*, **129**, 551–560.
- Siomi,M.C., Eder,P.S., Kataoka,N., Wan,L., Liu,Q. and Dreyfuss,G. (1997) Transportin-mediated nuclear import of heterogeneous nuclear RNP proteins. *J. Cell Biol.*, **138**, 1181–1192.
- Siomi,M.C., Fromont,M., Rain,J.-C., Wan,L., Wang,F., Legrain,P. and Dreyfuss,G. (1998) Functional conservation of the transportin nuclear import pathway in divergent organisms. *Mol. Cell Biol.*, **18**, 4141–4148.
- Stade,K., Ford,C.S., Guthrie,C. and Weis,K. (1997) Exportin 1 (Crm1p) is an essential nuclear export factor. *Cell*, **90**, 1041–1050.
- Stahl,B., Chou,J.H., Li,C., Sudhof,T.C. and Jahn,R. (1996) Rab3 reversibly recruits rabphilin to synaptic vesicles by a mechanism analogous to raf recruitment by ras. *EMBO J.*, **15**, 1799–1809.
- Stolow,D.T. and Haynes,S.R. (1995) *Cabeza*, a *Drosophila* gene encoding a novel RNA binding protein, shares homology with *EWS* and *TLS*, two genes involved in human sarcoma formation. *Nucleic Acids Res.*, **23**, 835–843.
- Sudhof,T.C. (1997) Function of Rab3 GDP–GTP exchange. *Neuron*, **18**, 519–522.
- Sukegawa,J. and Blobel,G. (1993) A nuclear pore complex protein that contains zinc finger motifs, binds DNA and faces the nucleoplasm. *Cell*, **72**, 29–38.
- Takiguchi,S., Tokino,T., Imai,T., Tanigami,A., Koyama,K. and Nakamura,Y. (1993) Identification and characterization of a cDNA, which is highly homologous to the ribonucleoprotein gene, from a locus (*D10S102*) closely linked to *MEN2* (multiple endocrine neoplasia type 2). *Cytogenet. Cell Genet.*, **64**, 128–130.
- Truant,R., Fridell,R.A., Benson,R.E., Bogerd,H. and Cullen,B.R. (1998) Identification and functional characterization of a novel nuclear localization signal present in the yeast Nab2 poly(A)⁺ RNA binding protein. *Mol. Cell Biol.*, **18**, 1449–1458.
- Visa,N., Alzhanova-Ericsson,A.T., Sun,X., Kiseleva,E., Björkroth,B., Wurtz,T. and Daneholt,B. (1996) A pre-mRNA-binding protein accompanies the RNA from the gene through the nuclear pores and into polysomes. *Cell*, **84**, 253–264.
- Wang,Y., Okamoto,M., Schmitz,F., Hofmann,K. and Sudhof,T.C. (1997) Rim is a putative Rab3 effector in regulating synaptic-vesicle fusion. *Nature*, **388**, 593–598.
- Wehner,E.P., Rao,E. and Brendel,M. (1993) Molecular structure and genetic regulation of *SFA*, a gene responsible for resistance to formaldehyde in *Saccharomyces cerevisiae* and characterization of its protein product. *Mol. Gen. Genet.*, **237**, 351–358.
- Weighardt,F., Biamonti,G. and Riva,S. (1995) Nucleo-cytoplasmic distribution of human hnRNP proteins: a search for the targeting domains in hnRNP A1. *J. Cell Sci.*, **108**, 545–555.
- Weis,K., Ryder,U. and Lamond,A.I. (1996) The conserved amino-terminal domain of hSRP1 α is essential for nuclear protein import. *EMBO J.*, **15**, 1818–1825.

- Wilken,N., Senecal,J.-L., Scheer,U. and Dabauvalle,M.-C. (1995) Localization of the Ran-GTP binding protein RanBP2 at the cytoplasmic side of the nuclear pore complex. *Eur. J. Cell Biol.*, **68**, 211–219.
- Wu,J., Matunis,M.J., Kraemer,D., Blobel,G. and Coutavas,E. (1995) Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain and a leucine-rich region. *J. Biol. Chem.*, **270**, 14209–14213.
- 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.
- Yokoyama,N. *et al.* (1995) A giant nucleopore protein that binds Ran/TC4. *Nature*, **376**, 184–188.

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