Nuclear transport: what a kary-on!

Steven J Gamblin* and Stephen J Smerdon

Compartmentalisation in eukaryotic cells presents special problems in macromolecular transport. Here we use the recently determined X-ray structures of a number of components of the nuclear transport machinery as a framework to review current understanding of this fundamental biological process.

Address: The Division of Protein Structure, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

*Corresponding author.
E-mail: s-gambli@darwin.nimr.mrc.ac.uk

Structure September 1999, **7**:R199–R204
http://biomednet.com/elecref/09692126007R0199

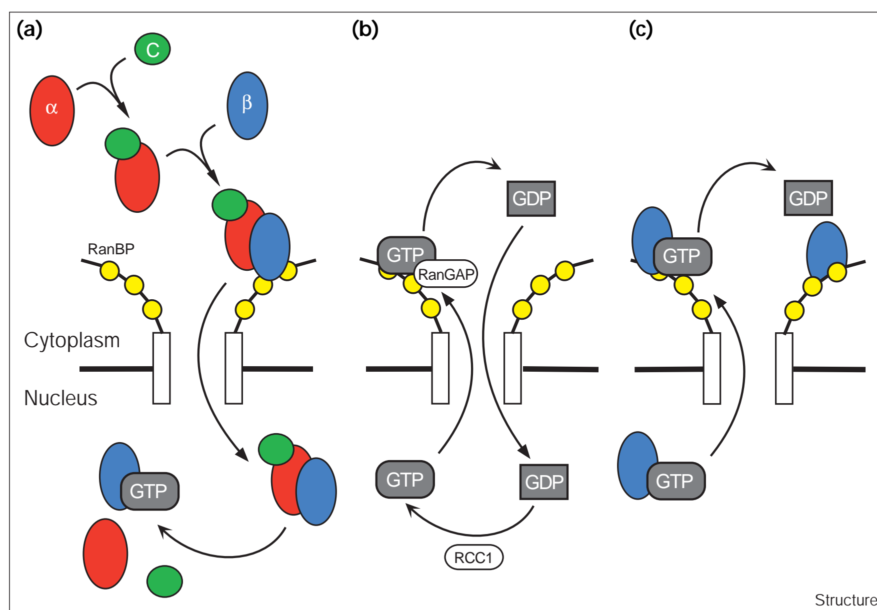
0969-2126/99/$ – See front matter
© 1999 Elsevier Science Ltd. All rights reserved.

Introduction

Eukaryotic cells are defined by the presence of a distinct, nuclear compartment that is enveloped by a double lipid bilayer. Therefore, specific machinery is required to permit the movement of more than a million macromolecules of cargo per minute between the nucleus and the cytoplasm. Bidirectional transport is achieved by means of huge macromolecular assemblies called nuclear pore complexes (NPCs) that can have molecular weights in excess of 10^8 Da. NPCs penetrate the nuclear membrane creating an aqueous channel through which traffic is actively passed in both directions [1]. Transport through the NPC is mediated by the karyopherin superfamily of proteins (also known as importins). Functionally, these molecules can be divided into subgroups depending on whether they mediate import to (importins) or export from (exportins) the nucleus. These molecules provide the means of recognising the nuclear localisation sequences (NLS) and nuclear export sequences (NES) of target proteins. Karyopherins also interact with the translocation apparatus of the NPC. Thus, they may be regarded as adaptor molecules between their cargoes and the nuclear pore. Once the karyopherin has transported its cargo it must be returned across the nuclear membrane to enable subsequent transport cycles.

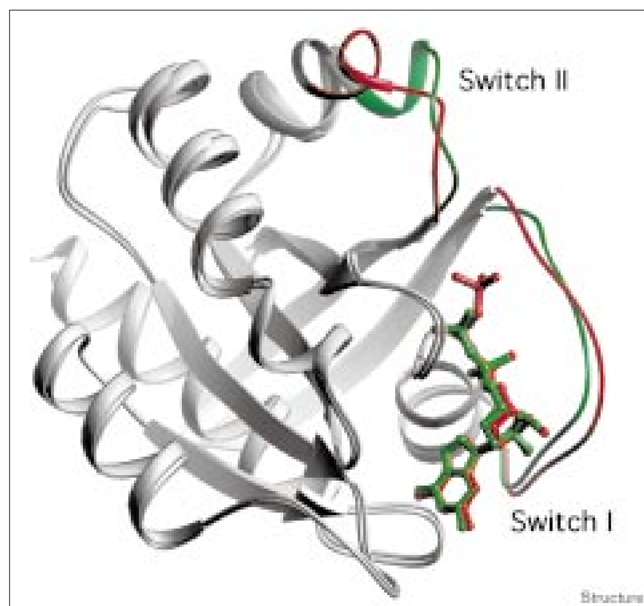
The karyopherin cycle (Figure 1) is coupled to, and driven by, the GTPase cycle of the small G protein Ran [2]. All small G proteins function by having two distinct conformations depending on whether they are bound to GTP or GDP. They are said to be in their active state when GTP-bound, because this conformation allows them to interact with their downstream effectors. Hydrolysis of bound GTP and the loss of the high-energy γ-phosphate leads to conformational changes in the so-called switch regions (Figure 2). Like many other small G proteins, Ran has a slow intrinsic rate of GTP

Figure 1



Schematic representation of the import cycle. **(a)** The assembly of the importin–cargo complex in the cytoplasm. The components of the system are coded: C, cargo (green); α, α-karyopherins (red); β, β-karyopherins (blue). Following transport through the NPC, the complex is disrupted by binding of Ran•GTP (grey) to the β-karyopherin. **(b)** The GTPase cycle of Ran indicating how the asymmetric distribution of the GDP-bound (rectangular boxes) and GTP-bound (rounded boxes) forms is achieved by the action of RanGAP and RanGEF (RCC1). **(c)** β-karyopherin–Ran•GTP returns to the cytoplasmic side of the NPC where it binds to RanBPs (yellow) and GTP hydrolysis catalysed by RanGAP occurs.

Figure 2



Comparison of the GTP-bound (red) and GDP-bound (green) structures of the archetypal small GTPase, Ras. The superposition illustrates the conformational changes that occur in the switch I and II regions upon GTP hydrolysis.

hydrolysis. Ran is unique, however, in that the auxiliary proteins which catalyse the GTPase cycle — guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) — are distributed asymmetrically across the nuclear membrane. Specifically, the Ran GEF RCC1, which is responsible for generating Ran•GTP from Ran•GDP, is sequestered inside the nucleus through its interaction with chromatin [3]. Conversely, RanGAP, which catalyses GTP hydrolysis, is attached via the Ran-binding domains (RanBDs) of Ran-binding protein 1 (RanBP1) and RanBP2 to the cytoplasmic fibrils of the nuclear pore (Figure 1) [4]. This results in the location of the bulk of cellular Ran•GTP in the nucleus and Ran•GDP in the cytosol. It is this asymmetry of distribution that drives both nuclear import and nuclear export cycles, because Ran•GTP disrupts importin–cargo complexes but is necessary for the formation of exportin–cargo complexes. Therefore, nuclear transport is only indirectly coupled to the hydrolysis of GTP. Karyopherins respond specifically to the GTP conformation of Ran, but GTP hydrolysis is the cost that must be paid to ensure that there is very little Ran•GTP in the cytosol.

In order to mediate vectorial nuclear transport karyopherins must meet three functional requirements. Firstly, they must be able to recognise and bind appropriate cargo molecules. Secondly, they must respond to nuclear Ran•GTP by either binding (exportins) or releasing (importins) cargo. Finally, they must interact with, and be transported through, the NPC. Several crystal structures have now been solved which add considerably to our understanding of the first two aspects of their biological activity (Table 1), but we still know relatively little about the interactions of karyopherins with the NPC. This review will be concerned only with nuclear import as components of the export machinery have yet to be structurally characterised.

Table 1

X-ray structures of nuclear transport proteins and their complexes.

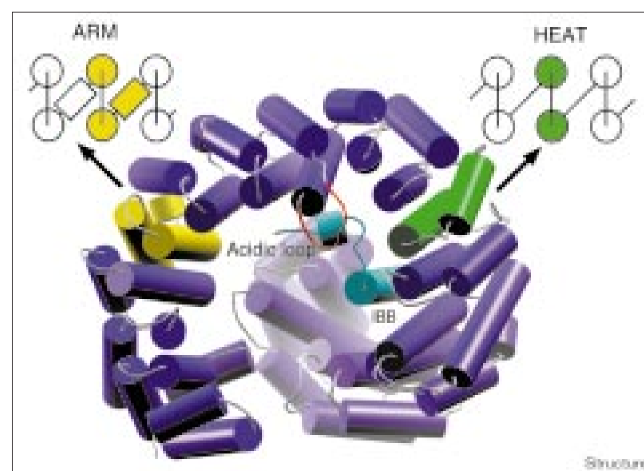
Protein/complex	PDB ID	Source	Reference
Ran•GMPPNP–importin-β fragment*	1IBR	Human	[8]
Ran•GMPPNP–karyopherin-β2*	1QBK	Human	[12]
Ran•GMPPNP–RanBD1*	1RRP	Human	[13]
Ran•GDP	1BYU	Dog	[14,15]
Ran•GDP–NTF2†	1A2K	Dog	[16]
Karyopherin-α	1BK5	Yeast (*Saccharomyces cerevisiae*)	[10]
Karyopherin-α–SV40 T antigen NLS	1BK6	Yeast (*Saccharomyces cerevisiae*)	[10]
Importin-α	1IAL	Mouse	[11]
Importin-β–IBB (crystal form I)	1QGK	Human	[9]
Importin-β–IBB (crystal form II)	1QGR	Human	[9]
RCC1 (RanGEF)	1A12	Human	[3]
Rna1p (RanGAP)	1YRG	Yeast (*Saccharomyces pombe*)	[4]

*GMPPNP, 5′-guanylyimidodiphosphate (a nonhydrolysable analogue of GTP). †NTF2, nuclear transport factor 2.

Karyopherins

A diverse range of proteins involved either directly or indirectly with mediating nuclear transport belong to the karyopherin superfamily. The α-karyopherins (also called importin-αs) contain eight 'armadillo' (ARM) repeats, each of about 40 residues, and function as adaptors between cargo molecules and the β-karyopherins. β-Karyopherins (also called importin-βs) contain approximately 15 'HEAT' motifs, each of which contain 35–45 residues, and are responsible for interaction with the NPC itself. ARM repeats contain three α helices and HEAT repeats only two [5]. Nevertheless, phylogenetic analysis strongly suggests that all karyopherins are evolutionarily related [6]. Comparison of 'generic' ARM and HEAT repeat structures does reveal differences in overall shape and helical packing [5,7], but they are similar in topology. Analysis of the helical repeats from importin-β has shown that they vary from canonical HEAT repeats to more ARM-like structures [8]. Karyopherins are thus made up from approximately parallel packing of multiple repeats of these building blocks; the blocks are packed in such a way as to produce a right-handed superhelix with a continuous hydrophobic core and two extended surfaces (Figure 3). β-Karyopherins thus possess an outer (convex) surface that is formed by the first helix in each repeat unit. The inner (concave) surface that constitutes the binding site for Ran and cargo molecules is made up from repeats of the second helix. In the case of α-karyopherins, the first two helices of each ARM repeat form the outer surface and the third helix forms the inner surface of the molecule. The available crystal structures of karyopherins and their complexes show that, although all karyopherins obey an underlying topological theme, there are substantial differences in tertiary structure. Presumably, these reflect differences in both primary sequence and binding partner.

Figure 3



The structure of importin-β bound to the IBB domain (turquoise) of importin-α [9]. Helices are represented as tubes. The overall architecture of the importin-β molecule is broadly representative of the karyopherin family as a whole and consists of a tandem array of ARM-like (yellow) and HEAT (green) helical repeat motifs. The extended helical bundle forms an extended superhelix, the axis of which is approximately normal to the page.

Cargo binding

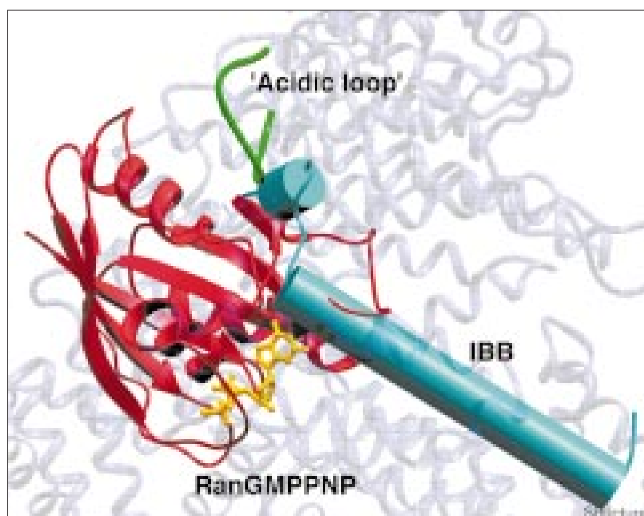
Importin-α interacts with importin-β through its N-terminal region, known as the importin-β binding domain (IBB). The structural basis of this interaction has now been revealed by the structure of importin-β–IBB peptide complexes [9]. Basic residues within the α-helical IBB domain make extensive contacts to an acidic surface in the C-terminal part of importin-β. The resulting compact, snail-like, appearance of importin-β (Figure 3) is probably a consequence of a substantial deformation of the superhelix upon IBB binding. This proposed change in superhelical structure may well be an important element in the Ran regulation of cargo binding.

Two other structures are pertinent to the cargo binding of karyopherins: a fragment of karyopherin-α, lacking its IBB domain but bound to an NLS peptide [10], and the structure of full-length importin-α [11]. In both cases the molecules adopt a similar, largely extended, superhelical structure. The IBB domain of importin-α contains an NLS-like sequence and about 11 of these residues bind in an extended fashion to the inner surface of importin-α. This interaction is very similar to the binding of NLS peptide to truncated karyopherin-α and suggests an autoregulatory activity [11]. Accordingly, binding of import cargo molecules to importin-α in the cytosol is favoured by the binding of importin-β to its IBB domain, thus preventing the IBB region from competing with the cargo. When the importin-α–importin-β–cargo complex passes into the nucleus and meets Ran•GTP, importin-β releases its grip on the IBB domain which is then free to compete with the binding of cargo to importin-α. By this means importin-α could exist in a state with high affinity for cargo in the cytosol, but in a low affinity form in the nucleus. Interestingly, a part of the IBB domain which is seen as an extended chain in the full-length importin-α structure [11] adopts a helical conformation in the importin-β–IBB structure.

Binding to Ran

The structures of Ran in complex with two different karyopherins (importin-β and karyopherin-β; Table 1) show that Ran (in its GTP-bound conformation) binds towards the N-terminal half of the molecule and brings about, at least local, changes in the conformations of both proteins [8,12]. Both of these structures also show important interactions of Ran with the so-called acidic loops of the karyopherins. Although these loops are of quite different length in the two structures, they are both located approximately in the middle of the protein. Unfortunately, the mechanism by which Ran displaces the IBB domain from its binding site cannot be assessed directly as at present no structure of Ran

Figure 4



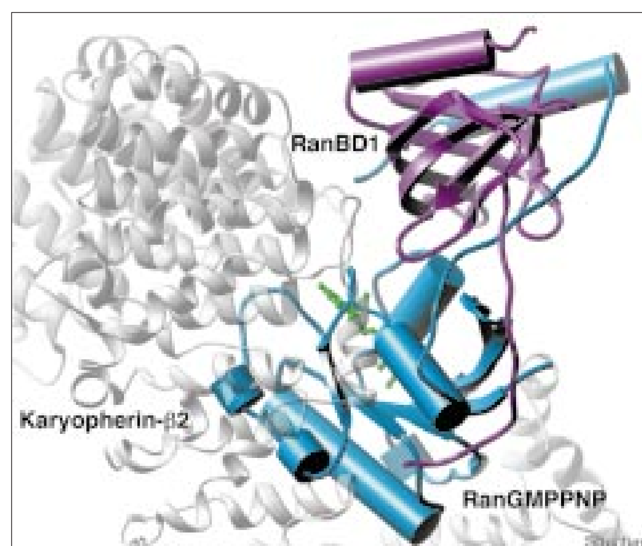
The overlap of regions of importin-β common to two complex structures (importin-β fragment–Ran•GMPPNP [8] and importin-β–IBB [9]) allows the G protein to be crudely positioned in the importin-β–IBB complex. The IBB peptide interacts with the acidic loop (green) but clashes with Ran, indicating a plausible mechanism for cargo displacement by G-protein binding.

bound to full-length importin-β is available. Chook and Blobel [12] have suggested ways in which the G-protein binding status of the karyopherin may be communicated to the cargo-binding site by means of the acidic loop. However, comparison of the Ran•GMPPNP–importin-β fragment structure with that of full-length importin-β–IBB suggests an alternative model. Superposition of the regions of importin-β that are common to both complexes allows Ran to be positioned in the importin-β–IBB structure (Figure 4). This results in substantial steric clashes between Ran and parts of both the IBB domain and the C-terminal portion of the importin-β molecule, thus indicating a rather more straightforward mechanism in which Ran binding directly displaces cargo from importin-β. As mentioned above, the acidic loops of both importin-β and karyopherin-β seem to be particularly important in mediating their interactions with Ran. In this light, it is interesting that the superhelical curvature of karyopherin-β is markedly more open than the corresponding part of importin-β. Comparison of the two structures suggests that it may be the presence of a much larger acidic loop in karyopherin-β that causes this opening up of the superhelix. Again there is circumstantial evidence, both from these crystal structures and biochemical experiments, which suggests that flexibility within the superhelical arc of the karyopherins may well be important for their function.

Ran

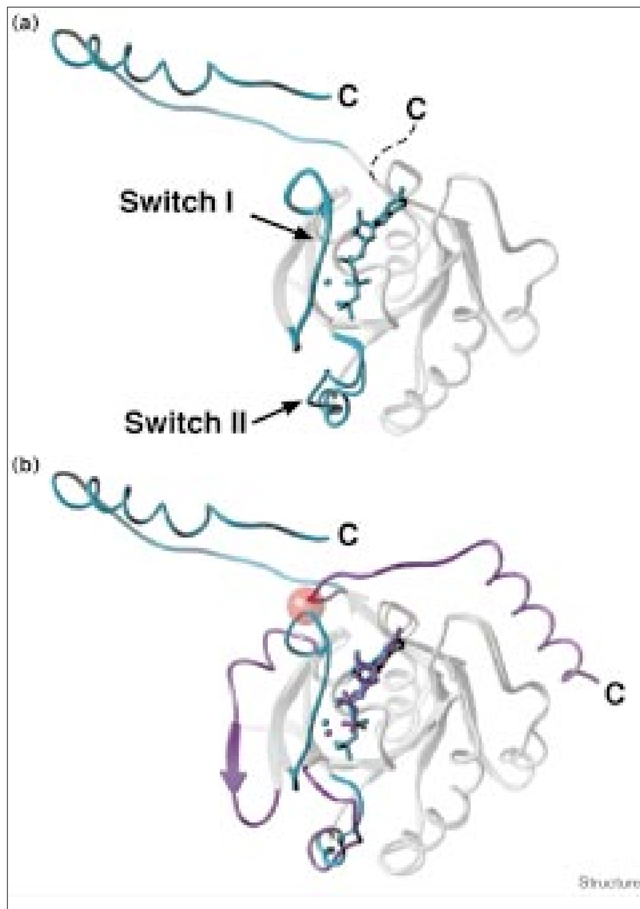
In contrast to the generic switch I and II regions, Ran appears unique amongst small G proteins in that it has an extended C terminus that is markedly acidic and the conformation of which is sensitive to the GTP/GDP status of the protein [13]. As there is a structure of Ran•GDP [14,15] but not of Ran•GTP alone, it has not been possible to directly define the conformational changes that occur as a result of GTP hydrolysis. However, given the structures of Ran bound to GMPPNP (a GTP analogue) in complex with importin-β [8], karyopherin-β2 [12] and RanBD1 [13] it is now possible to deduce the nature of this conformational switching. Importantly, Ran uses non-overlapping surfaces to form complexes with the RanBD and with the karyopherins (Figure 5). If the structures of Ran•GMPPNP– RanBD and Ran•GMPPNP–importin-β are compared (Figure 6a), they show that switch I is essentially identical in the two structures, switch II shows some local changes and the C terminus (from residue 178 of Ran onwards) is completely different. The fact that the switch I region is the same in both complexes gives confidence that this conformation is directed by the presence of GTP on the G protein. The conformational change in switch I between the GDP- and GTP-bound forms is substantial (Figure 6b). Two helical turns and a β strand are lost such that the final GTP-bound protein conforms reasonably to the canonical triphosphate structure of small G proteins. This analysis, in turn, adds further weight to the suggestion that the C terminus of Ran changes its conformation in response to the exchange of GTP for GDP [13]. In the GDP-bound form, the position of residues 182, 183 and 186 within the C-terminal region of Ran are inconsistent with the conformation of switch I seen in the GTP-bound state. Consequently, the C terminus of Ran adopts

Figure 5



Superposition of the Ran components of the Ran–karyopherin-β2 [12] and Ran–RanBD1 [8] complexes. RanBD1 and the karyopherin interact with rather different surfaces on the G protein.

Figure 6



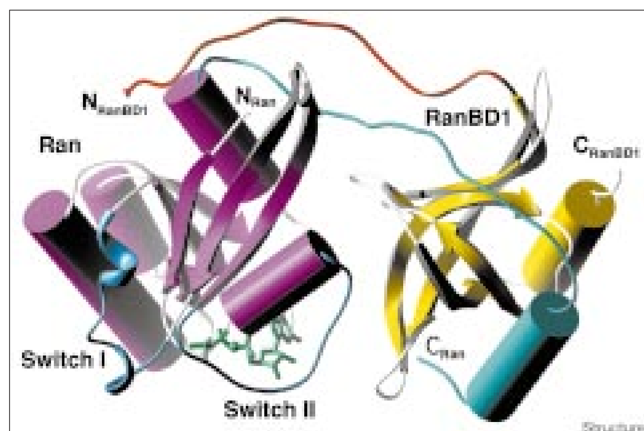
Comparison of the switch regions and C-terminal conformations in various 'GTP-' and GDP-bound forms of Ran. **(a)** Superposition of the GMPPNP-bound Ran structures as seen in the complexes with RanBD1 and karyopherin-β2 [9,13]. The switch I and II regions, together with the C-terminal region from the Ran–RanBD1 complex, are highlighted in blue. Although some differences occur in switch II, the 'GTP'-bound conformations of switch I are nearly identical. The C terminus of Ran in the karyopherin-β2 complex is disordered and represented by a dotted line. **(b)** Superposition of GMPPNP-bound Ran [13] with that of Ran•GDP [14,15]. In the GDP-bound form (purple) the C terminus associates with a basic patch on the G-protein surface. The change in the switch I conformation upon GTP binding is substantial and induces an equally dramatic alteration in the C-terminal region to avoid steric clashes [13] (pink sphere).

a different conformation upon GTP binding in order to avoid a steric clash with the GTP-bound conformation of switch I. Various biochemical data suggest that the effect of binding GTP to the G protein is to weaken, but not to abolish, the interaction of the C-terminal helix with the 'basic patch' on Ran (residues 139–142) [13]. Of all the Ran structures so far determined, none show well-defined electron density for the Asp-Glu-Asp-Asp-Asp-Leu (DEDDDL) motif at the extreme C terminus. This is rather surprising as several biochemical experiments demonstrate the functional importance of this motif. It appears that a nonspecific electrostatic interaction between the acidic motif and the basic patch must be invoked in order to explain interactions that do not result in a specific C-terminal conformation. Overall, this third C-terminal switch region seems to be particularly important for the complexes that Ran forms with its effectors, be they karyopherins or RanBDs. Formation of each of these complexes involves the effector molecules providing a so-called acidic loop, which takes the place occupied by the C terminus of Ran in its GDP-bound form. In complex with karyopherins the Ran C-terminus remains disordered, whereas in RanBD it makes extensive contacts with the effector (see below).

GTPase activity of Ran and its complexes

Binding of importin-β to Ran•GTP suppresses both its intrinsic and GAP-stimulated GTP hydrolysis activity. This inhibition is crucial for the biological function of Ran. The binding of Ran•GTP to importin-β in the nucleus dissociates bound cargo. In order to achieve vectorial transport, it is necessary that the GTP is not turned over until the importin-β has been exported from the nucleus. Having been transported to the cytoplasm it is then essential to dissociate the complex and hydrolyse the GTP. The recent crystal structures, together with biochemical data, offer possible explanations for both phenomena. Firstly, it is suggested that binding of Ran to importin-β or to karyopherin-β2 restricts access of RanGAP to the G protein and hence prevents GAP activity [8]. Secondly, it appears that the interaction of karyopherins with Ran may directly prevent GTP hydrolysis as follows. Small G proteins contain a highly conserved glutamine residue (Gln61 in Ras and Rho, Gln69 in Ran) within the switch II region that has an important mechanistic role in GTPase activity. This residue interacts with a water molecule and positions it appropriately for nucleophilic attack on the γ-phosphate phosphorous atom. Binding of Ran•GMPPNP to importin-β results in a change in conformation of Gln69 compared with that observed in the Ran•GMPPNP–RanBD1 complex, such that it is no longer able to interact with the hydrolytic water molecule [8]. A similar, non-productive conformation is seen for Gln69 of Ran in its complex with karyopherin-β2, although the detailed structure of switch II and its contacts with the effector are different in the two cases. Thus, the Ran–importin-β complex remains locked in the GTP state until it encounters RanBD at the nuclear pore exit. The RanBD binds tightly to Ran•GTP (K_d ~ 4 nM) and, as described previously, makes extensive contacts with the C terminus of Ran (Figure 7). Wittinghofer and colleagues propose that the binding of the RanBD to Ran–importin-β acts to dissociate importin-β from Ran, enabling RanGAP to bind and accelerate GTP hydrolysis [8]. Ran must then be transported back into the nucleus where it will be re-charged with GTP by the action of RCC1.

Figure 7



The structure of Ran•GMPPNP bound to the isolated RanBD1 fragment of RanBP2 [13]. An extensive interface is formed as the N-terminal region of RanBD1 wraps around the G protein. Similarly, the C-terminal segment of Ran embraces its binding partner through interactions that appear to require the conserved Asp-Glu-Asp-Asp-Asp-Leu motif. This motif, nevertheless, is disordered in this and all other Ran structures determined thus far.

The future

This review has described some of the recent developments in the structural biology of nuclear transport. Impressive as this work is, it is undoubtedly only the beginning. One of the more pressing questions relates to how Ran drives the dissociation of importin–cargo complexes, whilst constituting an essential component in the formation of exportin–cargo complexes. Answering this question in full will require determination of the crystal structures of several importins and exportins each in different bound states. This redundancy is necessary because the biochemistry of these systems strongly suggests important differences in the way members of each family function. Looking beyond the soluble components of nuclear trafficking, we have only begun to probe the structural basis of NPC function. For example, little is known about how karyopherins are recognised by the NPC. Furthermore, it is still unclear how the NPC accommodates the movement of transport complexes, some of them greater in size than the aqueous pore itself, from one side of the nuclear membrane to the other. Although the NPC itself is far too large for direct crystallographic analysis, the prospects for combining cryo-electron microscopy data with the crystal structures of isolated components are real and exciting. Using these approaches it should be possible, in time, to build up a rather detailed description of the process of nucleocytoplasmic transport.

Acknowledgements

We would like to thank K Rittinger, J Spencer and G Dodson for critical reading of this manuscript. Given the space limitations there are many important papers that have not been referred to directly, but we hope that most are included within the primary structure references and review papers.

References

1. Davis, L.I. (1995). The nuclear pore complex. *Annu. Rev. Biochem.* **64**, 865-896.
2. Mattaj, I.W. & Englmeier, L. (1998). Nucleocytoplasmic transport: the soluble phase. *Annu. Rev. Biochem.* **67**, 265-306.
3. Renault, L., *et al.,* & Wittinghofer, A. (1998). The 1.7 Å crystal structure of the regulator of chromosome condensation (RCC1) reveals a seven-bladed propeller. *Nature* **392**, 97-101.
4. Hillig, R.C., Renault, L., Vetter, I.R., Drell, T IV, Wittinghofer, A. & Becker, J. (1999). The crystal structure of rna1p: a new fold for a GTPase-activating protein. *Mol. Cell* **6**, 781-791.
5. Stewart, M. & Rhodes, D. (1999). Switching affinities in nuclear trafficking. *Nat. Struct. Biol.* **6**, 301-304.
6. Malik, H.S., Eickbush, T.H. & Goldfarb, D.S. (1997). Evolutionary specialisation of the nuclear targeting apparatus. *Proc. Natl Acad. Sci.* **94**, 13738-13742.
7. Groves, M.R. & Barford, D. (1999). Topological characteristics of helical repeat proteins. *Curr. Opin. Struct. Biol.* **9**, 383-389.
8. Vetter, I.R., Arndt, A., Kutay, U., Gorlich, D. & Wittinghofer, A. (1999). Structural view of the Ran–importin beta interaction at 2.3 Å resolution. *Cell* **97**, 635-646.
9. Cingolani, G., Petosa, C., Weis, K. & Muller, C.W. (1999). Structure of importin-β bound to the IBB domain of importin-α. *Nature* **399**, 221-229.
10. Conti, E., Uy, M., Leighton, L., Blobel, G. & Kuriyan, J. (1998). Crystallographic analysis of the recognition of a nuclear localisation signal by the nuclear import factor karyopherin α. *Cell* **94**, 193-204.
11. Kobe, B. (1999). Autoinhibition by an internal nuclear localisation signal revealed by the crystal structure of mammalian importin alpha. *Nat. Struct. Biol.* **4**, 388-397.
12. Chook, Y.M. & Blobel, G. (1999). Structure of the nuclear transport complex karyopherin-β 2-Ran•GppNHp. *Nature* **399**, 230-237.
13. Vetter, I.R., Nowak, C., Nishimoto, T., Kuhlmann, J. & Wittinghofer, A. (1999). Structure of a Ran-binding domain complexed with Ran bound to a GTP analogue: implications for nuclear transport. *Nature* **398**, 39-46.
14. Stewart, M., Kent, H.M. & McCoy, A.J. (1998). 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.
15. Scheffzek, K., Klebe, C., Fritz-Woolf, K., Kabsch, W. & Wittinghofer, A. (1995). Crystal structure of the nuclear Ras-related protein Ran in its GDP-bound form. *Nature* **374**, 378-381.
16. Stewart, M., Kent, H.M. & McCoy, A.J. (1998). 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.