CHAPTER SIX

Nuclear Pore Complex: Biochemistry and Biophysics of Nucleocytoplasmic Transport in Health and Disease

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Contents

Abstract

Nuclear pore complexes (NPCs) are the gateways connecting the nucleoplasm and cytoplasm. This structures are composed of over 30 different proteins and 60–125 MDa of mass depending on type of species. NPCs are bilateral pathways that selectively control the passage of macromolecules into and out of the nucleus. Molecules smaller than 40 kDa diffuse through the NPC passively while larger molecules require facilitated transport provided by their attachment to karyopherins. Kinetic studies have shown that approximately 1000 translocations occur per second per NPC. Maintaining its high selectivity while

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allowing for rapid translocation makes the NPC an efficient chemical nanomachine. In this review, we approach the NPC function via a structural viewpoint. Putting together different pieces of this puzzle, this chapter confers an overall insight into what molecular processes are engaged in import/export of active cargos across the NPC and how different transporters regulate nucleocytoplasmic transport. In the end, the correlation of several diseases and disorders with the NPC structural defects and dysfunctions is discussed.

Key Words: Nuclear pore complexes, Nucleocytoplasmic transport, Karyopherins, Importin, Exportin, Ran, Infectious disease, Cancer.

1. Introduction

The nucleus and cytoplasm are home to a myriad of processes vital to the cell. These processes are dependent upon shuttling of various macromolecules between these two environments (Kau et al., 2004). Such transport phenomena occur through nanopores called nuclear pore complexes (NPCs). NPCs are embedded in the nuclear envelop (NE), where the internal and external membranes fuse (Fig. 6.1) (Peters, 2009a).

Different studies have shown that the NPC structure resembles an hourglass (Peters, 2009a) or a donut (Stoffler et al., 2003) shape but has an octagonal radial symmetry around its central axis (Frenkiel-Krispin et al., 2010; Miao and Schulten, 2009; Wolf and Mofrad, 2008; Yang and Musser, 2006) as well as a pseudo-twofold symmetry across the NE (Frenkiel-Krispin et al., 2010; Miao and Schulten, 2009). This structure comprises eight centered cylindrical frameworks, each called a spoke, which enclose a central channel. This channel is sandwiched between the cytoplasmic and nuclear rings (Fig. 6.1; Akey and Radermacher, 1993; Wolf and Mofrad, 2008). In addition, eight cytoplasmic filaments emanate from the cytoplasmic ring to the cytoplasm and eight nuclear filaments, which are branched from the nuclear ring, join each other in the nuclear side of the NPC, shaping a basket-like structure known as the nuclear basket (Fig. 6.1; Elad et al., 2009). Various microscopic studies have shown that the overall length of the NPC including its extended cytoplasmic and nuclear filaments reaches 150–200 nm, while its external diameter is around 100–125 nm. The radius of the cytoplasmic and nuclear sides of this channel is 60–70 nm, and it narrows to about 25–45 nm at the center (Adam, 2001; Brohawn and Schwartz, 2009; DeGrasse et al., 2009; Kau et al., 2004; Schwartz, 2005; Stoffler et al., 2003; Yang and Musser, 2006) (Fig. 6.1). Nevertheless, the opening diameter of this channel has been estimated to be approximately 10 nm (Shulga and Goldfarb, 2003). In addition to the central channel, some peripheral channels with diameters of about 8 nm reportedly exist,

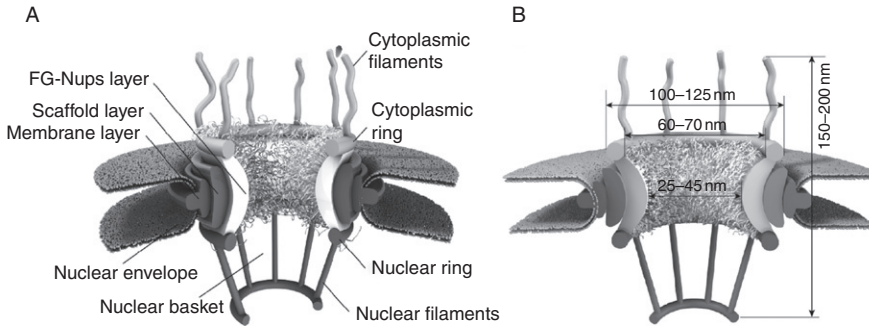

Figure 6.1 (A) The NPC structure resembles an hourglass (Peters, 2009a) or donut (Stoffler et al., 2003). This channel is sandwiched between the cytoplasmic and nuclear rings (Akey and Radermacher, 1993; Wolf and Mofrad, 2008). In addition, eight cytoplasmic filaments emanate from the cytoplasmic ring to the cytoplasm and eight nuclear filaments, which are branched from the nuclear ring, join each other in the nuclear side of the NPC, shaping a basket-like structure known as the nuclear basket (Elad et al., 2009). The structure of the NPC is composed of three concentric layers (Lusk et al., 2007; Suntharalingam and Wente, 2003; Walde and Kehlenbach, 2010): (1) The FG repeat layer is the innermost layer of the NPC and is directly exposed to cargo undergoing transport. (2) The membrane layer is the outermost layer that anchors the NPC to the NE. (3) The scaffold layer is located between the above-mentioned layers, forming the structure of the NPC (Lusk et al., 2007). (B) The overall length of the NPC including its extended cytoplasmic and nuclear filaments reaches 150–200 nm, while its external diameter is around 100–125 nm. The radius of the cytoplasmic and nuclear sides of this channel are 60–70 nm, with the radius narrowing to about 25–45 nm at the center (Adam, 2001; Brohawn and Schwartz, 2009; DeGrasse et al., 2009; Kau et al., 2004; Schwartz, 2005; Stoffler et al., 2003; Yang and Musser, 2006).

which contribute to transport of ions and small proteins (Frenkiel-Krispin et al., 2010; Kramer et al., 2007; Stoffler et al., 2003).

The NPC, as the largest nanomachine of the cell, is a bilateral, selective filter for a variety of molecules, transporting them quite rapidly without completely opening or closing the gateway structure (Kapon et al., 2008). Small molecules and ions ($M_r \sim 20$–40 kDa, diameter ~ 5–9 nm) travel through the NPC via passive diffusion, whereas larger molecules ($M_r > 40$ kDa up to $\sim$25 MDa, diameter of up to $\sim$40 nm) such as ribosomes, RNAs, and some proteins can only be transported through an active mechanism regulated by transporters (Miao and Schulten, 2009; Yang and Musser, 2006). It is observed that the size of the entering molecule affects the transport rate through the NPC (Moussavi-Baygi et al., 2011). A sharp drop in the transport rate is expected for cargo complexes with radii larger than the channel radius (Peters, 2005). Some researchers reported that passive diffusion and facilitated transport are not coupled and occur through different pathways (Naim et al., 2007). Nevertheless, recent studies provided evidence to refute this hypothesis, suggesting that passive and

facilitated transports take place through the same channel in the NPC (Mohr et al., 2009). Studies of microscopy indicate that the NPC structure dynamically changes its conformation in response to chemical or physical effectors, such as alterations in calcium ion concentration, Co_2, and ATP in the cell environment (Erickson et al., 2006; Oberleithner et al., 2000; Rakowska et al., 1998).

Proteomics analyses show that the NPC is a supra molecule made up of approximately 30 types of proteins named nucleoporins (Nups) (Fig. 6.2) (Hetzer, 2010; Walde and Kehlenbach, 2010). Due to the eightfold symmetry of the NPC structure, Nups have been observed in sets of multiple (8–48) copies in yeast and mammalian cells (Bednenko et al., 2003b). Therefore, the total number of Nups per NPC is estimated to be approximately 500–1000 (Peters, 2005; Sorokin et al., 2007). Some Nups are symmetrically found in both cytoplasmic and nuclear sides, some are only present on one side, and others make up the central framework (Strawn et al., 2004; Zeitler and Weis, 2004). This structure also possesses some mobile Nups that have different activities during each period of the cell cycle (Hou and Corces, 2010; Terry and Wente, 2009; Walde and Kehlenbach, 2010). The structure of the NPC is composed of three concentric layers (Fig. 6.1; Lusk et al., 2007; Suntharalingam and Wente, 2003; Walde and Kehlenbach, 2010): (1) The *FG repeat layer* is the innermost layer

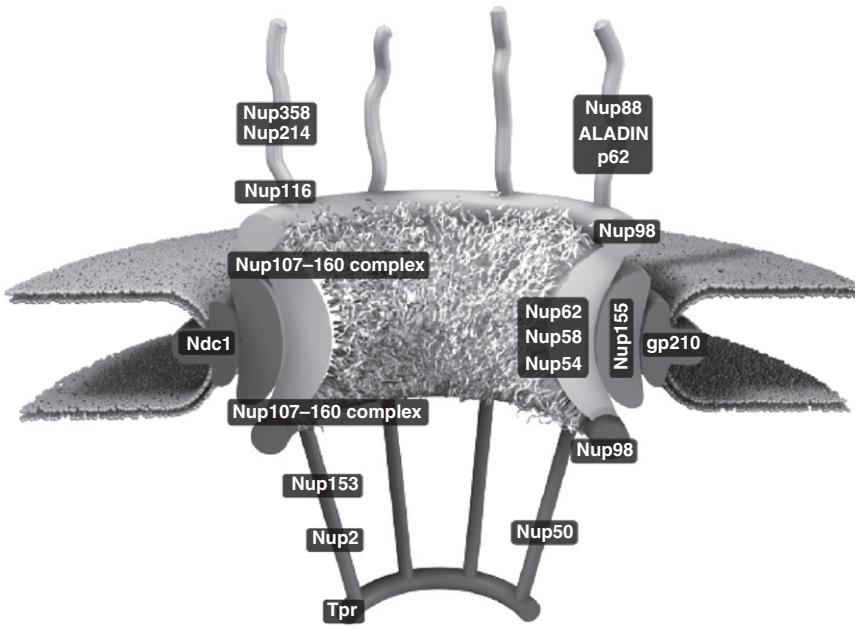

Figure 6.2 The overall structure of NPC along with some important Nups shown at their approximate positions.

of the NPC and is directly exposed to cargo undergoing transport; this layer coats the channel, facilitating the active transport of cargos (Terry and Wente, 2009). (2) The *membrane layer* is the outermost layer that anchors the NPC to the NE. (3) The *scaffold layer* is located between the above-mentioned layers, forming the structure of the NPC (Lusk et al., 2007). These layers are composed of various Nups (Fig. 6.2). The first layer is composed of phenylalanine–glycine-rich repeat domains such as FxFG, GLFG, PxFG, and SxFG. These Nups have flexible structures and are spread over the peripheral and central parts of the NPC (Denning et al., 2003; Peleg and Lim, 2010; Zeitler and Weis, 2004) (Fig. 6.1). It is reported that FG domains include some one-third of the Nups, and FG repeat domains account for 12–20% of the NPC mass (Devos et al., 2006; Frey and Gorlich, 2007). Although the cargo complex transport mechanism through the NPC remains unsolved, several models, such as virtual gate, selective phase, reduction of dimensionality, reversible FG, and forest model (Frenkiel-Krispin et al., 2010; Moussavi-Baygi et al., 2011; Peleg and Lim, 2010; Suntharalingam and Wente, 2003), have been proposed, each elucidating a transport mechanism through this proteinaceous structure in a distinct way.

The flexible domains of these Nups play a key role in the passage of cargos along the nucleotransport pathway via their low affinity with cargo. Molecules that travel through the NPC by binding to FG repeats have significantly higher transport rates than those without attachment to FG repeats, given their similar size and shapes (Ribbeck and GoÈrlich, 2001). Additionally, FG-Nups are equipped with coiled coil domains, which anchor them to the NPC bulk (Devos et al., 2006). The membrane layer of the NPC is composed of integral membrane proteins, which have transmembrane helices. Generally, these structures are equipped with α-helical domains to anchor them to the NE and with Cadherin-fold domains to reinforce the NE against excessive lateral movements. The only nondynamic Nups of the NPC are those existing in the membrane layer (Bednenko et al., 2003b). Aside from Nups containing FG, coiled coil, and transmembrane domains, all other Nups are part of the scaffold (the third) layer and include approximately 1/2 of all Nups (Walde and Kehlenbach, 2010). These Nups have β-propeller and α-solenoid structures and a significant percentage of the NPC mass is composed of these Nups, with approximately one-third of Nups containing α-solenoid domains (Devos et al., 2006; Rout et al., 2000). In addition, some Nups include specific structural motifs such as "Zinc finger domains" and motifs connected to RNA (Cassola and Frasch, 2009; Yaseen and Blobel, 1999).

Generally, the number of NPCs on the NE is independent of the nucleus' surface area and DNA volume. The number of NPCs per nucleus varies significantly for different species, environmental conditions, cell activities, and periods of the cell life cycle (Gerace and Burke, 1988).

For example, yeast barely have 200 NPCs per nucleus, while the number of NPCs per nucleus for vertebrates is on average 2000–5000 (10–20 pores/μm^2) (Fabre and Hurt, 1997). Large nuclei of *Xenopus oocytes* are home to as many as 5×10^7 NPCs (over 60 pores/μm^2) (Gorlich and Kutay, 1999). While NPCs are seen to exist throughout the cell cycle, the number of these pores doubles in dividing cells during interphase and before mitosis and they reach a maximum number in the S-phase of the cell cycle (D'Angelo et al., 2006). It has also been shown that some hormones increase the number and density of NPCs (Miller et al., 1991). Apparently, an increased number of NPCs provides some cells with resistance to chemotherapy (Lim et al., 2008). Experiments clearly indicate an increase in the number of NPCs in embryonic stem cells (ES) as they differentiate into proliferative cardiomyocytes. Therefore, differentiation of embryonic stem cells could be traced to changes in the number of NPCs. Differentiation of ES in cardiac progeny is likely associated with the structural and functional remodeling of the NPC (Lim et al., 2008; Perez-Terzic et al., 2007). A comparison between isolated cardiomyocytes from heart and ES-originated cells indicates no difference in dimensions of the NPCs. Nonetheless, it is reported that the number of NPCs in cells isolated from the heart is greater than those originating from stem cells. Also, increased transport activity of the NPC is reportedly observed in stem cell-derived cardiomyocytes (Perez-Terzic et al., 2003).

The overall architecture of the NPC is conserved among the eukaryotic cells (Brohawn and Schwartz, 2009) and this conservation is established in the last eukaryotic common ancestor (DeGrasse et al., 2009). It is likely that the NPC has been a structural part of another organism like archea (Bapteste et al., 2005). Also, it is speculated that NPCs are the chimaeras from endomembrane and mitosis-related chromatin-associated proteins (Cavalier-Smith, 2010). Investigations conducted on yeast and mammalian cells indicate that functions and localization of their NPCs are similar even though their sequences are not exactly conserved (Bapteste et al., 2005; Kiseleva et al., 2004; Neumann et al., 2006; Yasuhara et al., 2009). Interestingly, a large number of Nups are conserved among all known eukaryotic cells, but many of them are specific to certain cells (Frenkiel-Krispin et al., 2010; Neumann et al., 2006). As a result, it is claimed that the overall shape and size of the NPC has been conserved through evolution (Alber et al., 2007; Yasuhara et al., 2009); however, some structural distinctions are observable among NPCs of different species (Elad et al., 2009). Structural analyses illustrate some differences in the location of Nups as well as their number of copies among the NPCs of different species. In contrast to related species, though, NPCs of distinct species have low structural homology (Frenkiel-Krispin et al., 2010).

Indeed, transport through the NPC and the NPC components provides a broad range of vital functions for the cell. Comparative proteomic analysis of

the NPC predicts some unexpected functions for this massive complex (Elad et al., 2009). Generally, this structure could affect indispensible cellular functions, such as gene expression, DNA damage and repair, aging, apoptosis, and even determination of cell differentiation and fate (Batrakou et al., 2009; Mishra et al., 2010; Nagai et al., 2008; Nakano et al., 2010; Yasuhara et al., 2007, 2009; Wolf and Mofrad, 2009). As a result, any structural defect or malfunction in this key regulator could cause different diseases or even death. Since understanding the NPC structure is essential to deciphering its function, many scientists have been engaged in structural studies of this large complex during the past few years and various approaches have been exploited to investigate the role of the NPC in different diseases with the hope to find remedial solutions. In this review, we first examine and discuss how different molecules are transported through the NPC while the dysfunctions caused by the NPC structural defects will be described at the end.

This review is composed of two major parts. In the first part (Sections 2 and 3), we will discuss briefly how different molecules (Fig. 6.3) orchestrate the exquisite process of nucleocytoplasmic transport (NCT). Since some cargos need to form complexes with specific molecules, termed transporters, in order to be able to pass through the NPC, we will first explain the definition of transporters along with signal sequences on the cargo transporter proteins required to identify a protein. Section 2.1 will focus mainly on the structure of important transporters, such as importin α (impα) and importin β (impβ), and interactions between cargos and these transporters, which lead to the formation of a nucleus entering cargo complex in the

Human	Yeast	Involve in	Mw(KD)	Shape	Human	Yeast	Involve in	Mw(KD)	Shape
Impα	Kap60	Cargo complex association/disassociation Karyopherin recycling	60		NTF2/ P10	NTF2/ P10	Ran cycle	28	
Impβ	Kap95	Cargo complex association/disassociation	95		RanGAP	Ran1	Ran cycle	58–60	
Nup50	Nup2	Cargo complex disassociation	50		RanBP2	-	Ran cycle	358	
CAS	Cse1	Karyopherin recycling	100		RanBP1	Yrb1	Ran cycle	23	
Ran	Gsp1	Cargo complex disassociation Ran cycle	24		RanGEF /RCC1	Prp20	Ran cycle	45	

Figure 6.3 Some important molecules of nucleocytoplasmic transport.

cytoplasm. In Section 2.2, we will follow the pathway of a typical active cargo through the NPC, while Section 2.3 will take a glance at some newly deciphered molecular structures, such as Ran, Nup50 (Nup2p), and their interaction with the entering cargo complexes, which leads to dissociation of the complex in the nuclear basket. Next, in Section 2.4, the recycling pathway of transporters to the cytoplasm and the molecular structure of cellular apoptosis susceptibility molecule (CAS), which is highly engaged in this process, will be explained. Section 2.5 will examine the Ran cycle (hydrolysis process of GTP of RanGTP and replacement of GTP with GDP in RanGDP), the molecules involved in this cycle, and their contribution to NCT. Finally, in Section 3, we conclude this part of the review by summarizing the nucleocytoplasmic pathway mechanism. The second part of this review is dedicated to diseases and disorders linked to NPC structural and functional defects, for example cancer, nervous system and autoimmune disorders, and cardiac and infectious diseases will be discussed.

2. What Drives Cargo Transport Through the NPC?

The major task of the NPC is control and regulation of the traffic of macromolecules into and out of the nucleus. When considering the bidirectional transport of cargos from the cytoplasm to the nucleus and vice versa, on average, as many as 1000 transports are observed through an NPC per second (Fahrenkrog et al., 2004; Peters, 2005), with this high-throughput rate of cargo translocation achieved via transporters. The most important category of transporters is known as the β karyopherin family, which we call the karyopherin family hereinafter for the sake of simplicity (Cook and Conti, 2010; Fiserova and Goldberg, 2010; Pemberton and Paschal, 2005; Peters, 2009b). Members of this family are engaged in regulation of the NE and NPC assembly as well as the replication phenomenon (Mosammaparast and Pemberton, 2004). Karyopherins are possibly conserved through evolution and they are developed from a common ancestor. While the molecular weight of karyopherins is quite similar, they share no more than 20% common sequences (Mosammaparast and Pemberton, 2004). These large proteins (~100 kDa) are generally divided into two groups: importins and exportins. As their names suggest, importins control import of materials from the cytoplasm to the nucleus, while exportins help macromolecules exit the nucleus. Currently, 14 members of the karyopherin family in yeast and 20 of them in human cells have been identified. In human cells, 10 of these macromolecules belong to importins; however, only two groups are known to have a significant contribution to the transport phenomena and are more commonly called impα and impβ.

Also, seven types of exportins have been discovered, which include Crm1, exp-t, and CAS. Some karyopherins such as importin13 in human and msn5p in yeast are involved in import and export of materials across the NPC (Chumakov and Prasolov, 2010; Dorfman and Macara, 2008; Mosammaparast and Pemberton, 2004; Pemberton and Paschal, 2005; Strom and Weis, 2001). Additionally, there are other transporters, such as Tap/Mex67 and Calreticulin, that play critical roles in the transport process, yet they do not belong to the karyopherin family (Holaska et al., 2001; Tartakoff and Tao, 2010; Wente, 2000).

Members of the karyopherin family have three major characteristics. First, they need to bind to cargos to carry them. Second, if they are to pass through the NPC they must interact with FG repeats, which line the inner face of the NPC. Finally, they need a supply of energy for continued transport, which is provided via interactions with GTP-bound Ran (Ran is a member of Ras family GTPase) (Moore and Blobel, 1994). This trait has a central effect on the regulation of cargo transport. The affinity of karyopherins to Ran varies among members of the karyopherin family. Some karyopherins bind to Ran molecules with high affinities ($k_d \sim$ nM), whereas some others have low affinities to Ran (Macara, 2001). Further, evidence exists on the affinity of karyopherins to GDP-bound Ran and it is known that the impβ–RanGDP bond is four orders of magnitude weaker than the impβ–RanGTP bond (Lonhienne et al., 2009). However, karyopherins are capable of attaching to cargos which facilitates their passage through the channel. Some results indicate collisions and interactions of import transporters with cytoplasmic compounds slow down movement of the transporters. Nonetheless, the binding of transporters to cargos disrupts these interactions and expedites the transport process (Wu et al., 2009). It has been reported that karyopherins undergo post-translational modification, which also helps regulate the import and export of cargos (Mosammaparast and Pemberton, 2004).

Importins and exportins bind to certain sequences of the cargo termed nuclear localization sequences (NLSs) and nuclear export sequences (NESs), respectively. NLSs are divided into classic and nonclassic groups. Classic NLSs (cNLSs) contain basic charged amino acids like arginine and lysine, as opposed to nonclassic NLSs (ncNLS), which lack basic amino acid residues. There are two types of classic NLSs: monopartites contain a single basic amino acid stretch, while bipartites possess a couple of those stretches. This sequence, for instance, looks like "^{126}PKKKRK^{132}" in Simian Virus40 (SV40) T antigen, forming a monopartite NLS. In nucleoplasmin, the NLS can be shown as "^{155}KRPAATKKAGQAKKKK^{170}," having two stretches of basic amino acids 10–12 amino acids apart from each other and forming a bipartite NLS (Fontes et al., 2000, 2003; Lam and Dean, 2010; Lange et al., 2007). It was observed recently that length of the linker between two basic amino acid groups may vary depending on amino acid

compositions and reach to even 29 amino acids (Lange et al., 2010). Exported cargos are decorated with NESs usually having Leu-rich or the hydrophobic amino acids. Computational alignment studies show that most NESs have sequences like the following: φ-$X_{(2–3)}$-φ-$X_{(2–3)}$-φ-X-φ (where φ is one of the hydrophobic amino acids, M, F, V, L, and J, and X could be any arbitrary amino acid) (Kutay and Güttinger, 2005; La Cour et al., 2004; Lui and Huang, 2009).

2.1. Cargo complex association

2.1.1. Importin β

Impβ is a 95-kDa flexible super-helix, which can take different conformations. Like other members of the karyopherin family, its flexibility facilitates the formation of complexes with different cargo sizes and shapes. It is capable of interacting reversibly with crucial transport molecules such as cargo, FG-Nups, and RanGTP (Cook et al., 2007) (Fig. 6.4). This super-helix is composed of 19 tandem HEAT repeats (Huntingtin, elongation factor 3 [EF3], protein phosphatase 2A [PP2A], and the yeast PI3-kinase TOR1) (Fig. 6.5). HEAT repeat motifs exist in structures of impβ, CAS, and other importins and exportins. These repeats only have about 20% of their sequences in common with each other. However, they are mostly similar in their N-terminus, which is a binding site for Ran. HEAT repeats include 39 amino acid motifs and are composed of antiparallel A and B α-helices (Fig. 6.5). These A and B helices build two C-like arches, which connect to each other by a turn. These α-helices are stacked so that they construct a spring-like helicoidal structure. A-helices construct the convex side of impβ, while its concave side is composed of B-helices (Lee et al., 2005; Stewart, 2006; Strom and Weis, 2001; Zachariae and Grubmuller, 2008).

In the free condition, impβ has an S-shaped open conformation. It then closes when attached to the importin β binding (IBB) domain of impα, RanGTP, and Nups (Fig. 6.4). In other words, its helical pitch reduces upon attachment, like a snake wrapping around its "prey." Simulations indicate that impβ is curved when bound to a ligand and it opens up and elongates once it is released (Bednenko et al., 2003a; Cingolani et al., 1999; Conti et al., 2006).

2.1.2. Importin α

Impα is a 55-kDa protein that acts as an adaptor to connect classic NLSs to impβ. It is decorated with Arm repeats (Armadillo), which are related to impβ-producer HEAT repeats (Goldfarb et al., 2004) (Fig. 6.6). The Arm repeat is a 40 amino acid motif composed of three α-helices called H1, H2, and H3 (Tewari et al., 2010). These α-helices are mounted together in a right-handed super-helix, building a banana-like shape (Fig. 6.6). Analyses show that impα is composed of three major parts. Its N-terminus is

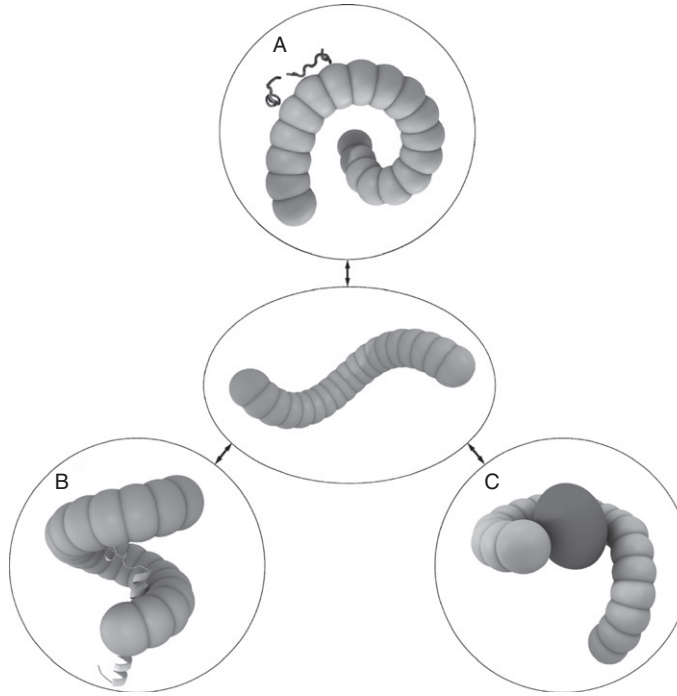

Figure 6.4 Impβ is a flexible super-helix that can take different conformations. This flexibility helps form complexes with a variety of cargo sizes and shapes. In its free condition (central figure), importin-β has an S-shaped open conformation. It closes when attached to Nups (A), the IBB domain of importin-α (B), or RanGTP (C). Its helical pitch reduces upon attachment, similar to a snake wrapping around its "prey" (Bednenko et al., 2003a; Cingolani et al., 1999; Conti et al., 2006).

composed of 40 amino acids referred to as the importin β binding (IBB) domain and is the impβ binding site (Fig. 6.6); its central part, which has Arm repeats, is able to bind to NLSs; and last, the C-terminus part with the 10th Arm repeat, which binds to CAS (Goldfarb et al., 2004; Stewart, 2006) (Fig. 6.6).

2.1.3. Interactions engaged in NLS–impα–impβ complex formation

The NLS–impα–impβ complex is known to assemble via two major processes. In the first mechanism, impα binds to the NLS and afterward to impβ. In the second process, which is actually more probable to occur, impα primarily binds to impβ and after that this complex binds to the NLS (Goldfarb et al., 2004). The IBB domain in impα has a basic L-shaped structure including an extended section bound to HEAT repeats 7–10 of impβ (Fig. 6.7). In this zone, the IBB interacts with an acidic loop

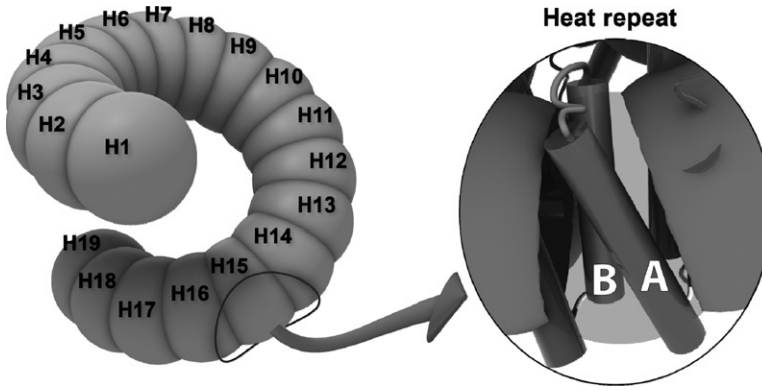

Figure 6.5 Importin-β: this super-helix is composed of 19 tandem HEAT repeats. HEAT repeat motifs exist in structure of impβ, CAS, and other importins and exportins. HEAT repeats include 39 amino acid motifs and are composed of antiparallel A and B α-helices. These A and B helices form two C-like arches, which connect to each other by a turn. These α-helices are piled so that they construct a spring-like helicoidal structure. A-helices construct the convex side of impβ, while its concave side is composed of B-helices (Lee et al., 2005; Stewart, 2006; Strom and Weis, 2001; Zachariae and Grubmuller, 2008).

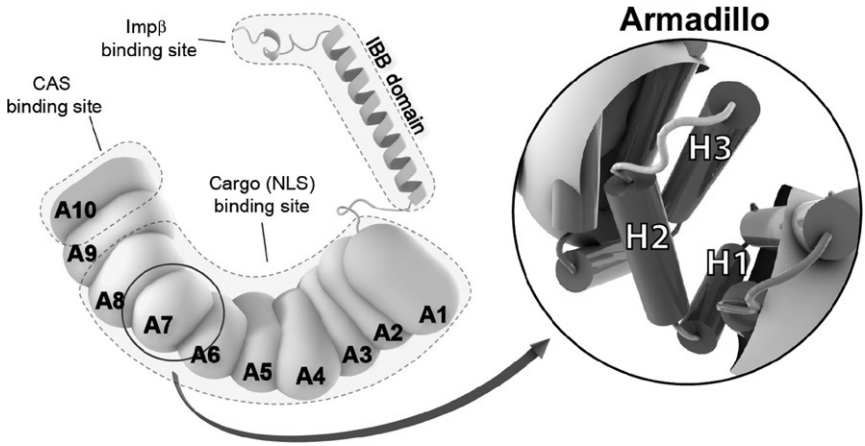

Figure 6.6 Impα is a 55-kDa protein that acts as an adaptor to connect classic NLSs to impβ. It is decorated with Arm repeats (Armadillo). Arm repeat is a 40 amino acid motif made up of three α-helices called H1, H2, and H3 (Tewari et al., 2010). These α-helices are stacked together in a right-handed super-helix, building a banana-like shape. The concave inner part of impα is made out of H3 α-helices. Analyses show that impα is composed of three major parts. First, the N-terminus part composed of 40 amino acids that is called IBB domain (importin β-binding domain) and is the impβ binding site. Second, the central part equipped with Arm repeats, which are able to bind to NLSs, and lastly, the C-terminus part with the 10th Arm repeat, which binds to CAS (Goldfarb et al., 2004; Stewart, 2006).

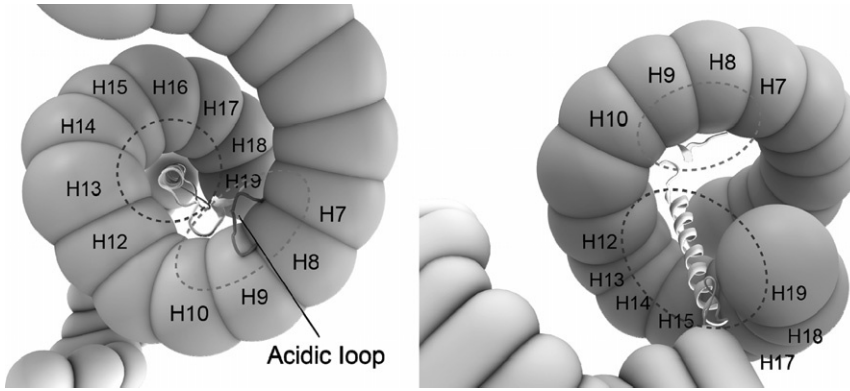

Figure 6.7 Importin α–β complex: the IBB domain in impα (lighter color) has a basic L-shaped structure including an extended section bound to HEAT repeats 7–10 of impβ (darker color). In this zone, the IBB interacts with an acidic loop located on the HEAT repeat 8. The IBB structure is also composed of α-helices attaching to HEAT repeats 12–19. The inner surface of impβ contains acidic residues that interact with positively charged IBB domain residues.

(DDDDDW) located on HEAT repeat 8 (Fig. 6.7). The IBB structure is also composed of α-helices attaching to HEAT repeats 12–19 (Fig. 6.7). In fact, the inner surface of impβ contains acidic residues that interact with positively charged IBB domain residues. As many as 40 different contacts including electrostatic, Van der Waals, and hydrophobic ones come together to attach the IBB domain to impβ (Cingolani et al., 1999; Madrid and Weis, 2006; Stewart, 2003; Zachariae and Grubmuller, 2008).

The concave inner part of impα comprises H3 α-helices (Fig. 6.6). This part has two binding sites for NLSs (Fig. 6.8). The first one is a major groove, which presents a larger area of binding between Arm repeats 2 and 4 and is closer to the impα N-terminus. The major groove attaches to the monopartite NLS and the larger basic cluster of the bipartite NLS. The second bond is the minor groove located between Arm repeats 7 and 8, forming a smaller area of binding (Fig. 6.8). This groove binds to the smaller cluster of the basic bipartite NLS residue and also to the monopartite NLS. Interaction between Asn, Trp, and acidic amino acids of impα with the basic ones of the NLS leads to this attachment (Fig. 6.8), and various sets of electrostatic, hydrophobic, and hydrogen bonds give rise to a firm impα–NLS attachment (Fontes et al., 2000; Yang et al., 2010).

The IBB domain has autoinhibitory characteristic, meaning that it competes with the NLS to attach to impα, and this is a result of resemblance of the NLS and the IBB domain amino acids. Typically, affinity of impα to the NLS is higher than its affinity to IBB domain; however, when the IBB is

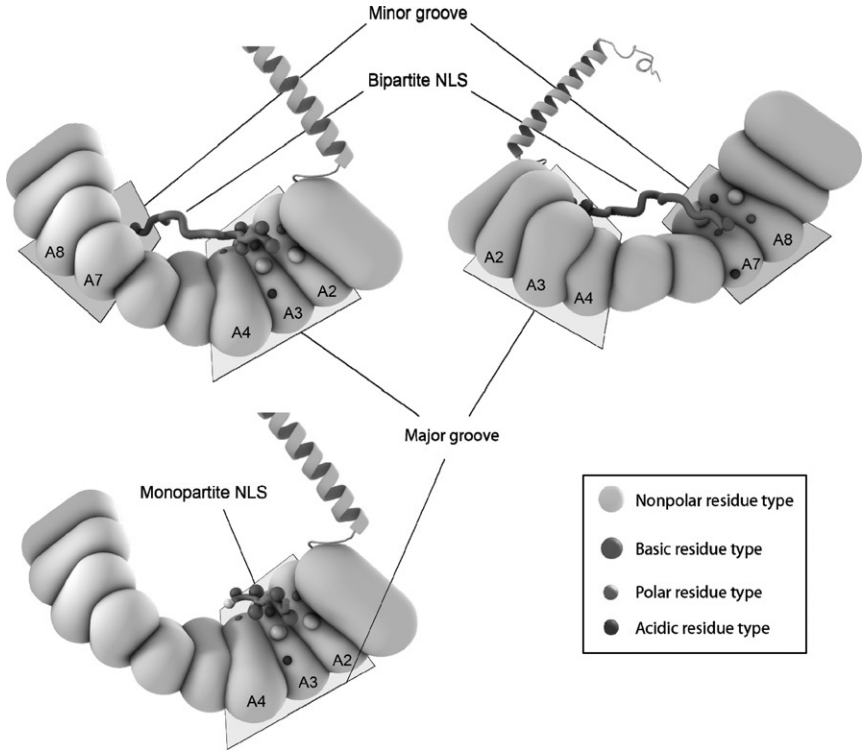

Figure 6.8 NLS–impα complex: the concave inner part of impα has two binding sites for NLSs. The first one is a major groove, which prepares a larger area of binding between Arm repeats 2 and 4, and is closer to the impα N-terminus. The major groove attaches to the monopartite NLS (bottom) and the larger basic cluster of the bipartite NLS (top). The second bond is the minor groove, located between Arm repeats 7 and 8, making a smaller area of binding. This groove binds to the smaller cluster of the basic bipartite NLS residue and also monopartite NLS. Interaction of Asn, Trp, and acidic amino acids of impα with the basic ones of NLS causes this attachment. Various sets of electrostatic, hydrophobic, and hydrogen bonds give rise to a firm impα–NLS attachment (Fontes et al., 2000; Yang et al., 2010).

attached to impβ, the NLS bond to impα is stronger and no autoinhibitory activity exists in the IBB domain. Interestingly, NLS affinity remains unchanged for impα without the IBB domain and impα bound to impβ (~10 nM). It seems that the entrance rate of cargos into the nucleus is correlated with NLS–impα bond strength (Catimel et al., 2001; Fanara et al., 2000; Lange et al., 2007; Stewart, 2007; Zilman et al., 2007). Impα has a homolog structure in mouse and yeast, and as there are different NLS types working in cells, the NLS recognition mechanisms should have been conserved (Conti and Kuriyan, 2000).

Despite the important role of the IBB domain in the formation of impβ–impα–NLS complex, some cargos carrying ncNLSs simply bind to impβ directly. These cargos, equipped with ncNLS motifs, resemble the IBB domain. For example, the structure of HIV-1, Rev, and Tat proteins includes Arg motifs that shape a helical secondary structure and imitate the IBB domain function (Truant and Cullen, 1999). It is yet to be determined if impβ includes a secondary binding site (other than the domain bound to the IBB) for impα-independent cargos, or if an impβ-targeting NLS facilitates forming a direct bond to impβ (Cingolani et al., 2002).

2.2. Transport of cargo complex across the channel

After the cargo–carrier complex is formed, it passes across the NPC channel. During the passage, FG-Nups, which are speculated to occlude the central channel, interact weakly with impβ (Fig. 6.9). This interaction with μM-range affinity is the key corner stone for the selective transport. Each FG-Nup is composed of 20–30 FG-rich domains, such as FG, GLFG, FxFG (where x could be any amino acid) (Fig. 6.9; Frey and Gorlich, 2007, 2009). In fact, FxFG repeats are separated from each other by Thr- and Ser-rich spacer sequences; however, spacer sequences between GLFG repeats have

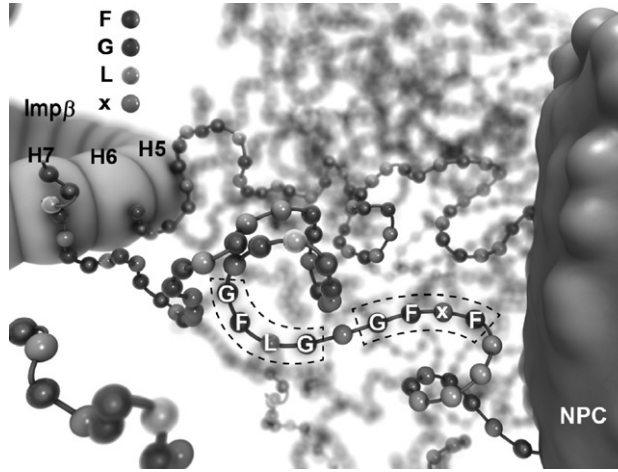

Figure 6.9 FG-Nups: after the cargo–carrier complex is formed, it passes through the NPC. During the passage, FG-Nups, which are speculated to occlude the central channel, interact weakly with imp-β. Each FG-Nup is composed of 20–30 FG-rich domains, such as FG, GlFG, FxFG (x could be any amino acid), and so on (Frey and Gorlich, 2007, 2009). FG-Nups hold hydrophobic interactions on the convex face of impβ. These Nups bind impβ somewhere between HEAT repeats 5 and 7 (Bayliss et al., 2002). Also, another binding site has been recognized on HEAT14-16 of importin-β.

abundant amounts of Gln and Asn and no acidic residues are observed in these sequences (Terry and Wente, 2009). GLFG and FxFG Nups bind to overlapping sites on Impβ, somewhere between HEAT repeats 5 and 7 (Fig. 6.9; Bayliss et al., 2002). FG-Nups are distributed over the internal face of the central channel as well as nuclear and cytoplasmic sides of the NPC. FG domains of these Nups have flexible structures, and some of them, such as Nup153 and Nup214, are even able to move from one side of the channel all the way to the other side of the channel (Paulillo et al., 2006). On average, 3500 FG repeats exist per NPC (Miao and Schulten, 2009; Strawn et al., 2004).

It is reported that removing up to 50% of FG motifs does not significantly affect the transport process or cell life. However, removing specific FG motifs, such as those in Nup116 (Fig. 6.2), would be fatal to the cell (Stewart, 2007; Strawn et al., 2004). Generally, removing the symmetric-about-NE-planar-axis FG-Nups does have a significant effect on transport. This is because certain combinations of FG domains of symmetric FG-Nups, especially GLFGs, are critical to transport. However, some types of transport processes remain unchanged even after removal of the symmetric FG-Nups, which clearly proves distinct transport pathways exist for different carriers (Strawn et al., 2004; Zeitler and Weis, 2004). Experimental studies and simulations show an attachment between impβ and Nups occurs via some hydrophobic binding spots, meaning that FG-Nups hold hydrophobic interactions on the convex face of impβ (Fig. 6.9). During the primary interaction, the hydrophobic side chain of Phe in GLFG and FxFG domains penetrates the hydrophobic pocket of impβ involving HEAT repeats 5–7. Additionally, another FG-Nup binding site has been recognized on HEAT repeats 14–16 of impβ (Bednenko et al., 2003a; Zeitler and Weis, 2004). Experiments show that bonds between FG domains and transporters are sensitive to the number of FG motifs and amino acids located right after them. In other words, the tendency of each FG-Nup to interact with a carrier protein depends on how many FG repeats exist in its proximity, while interaction strength might be modulated by the linkers between the consecutive FG-Nups (Patel and Rexach, 2008; Stewart, 2007). It turns out that Phe located in FG motifs plays the major role in making the connection between impβ and FG repeats. Substituting Phe on an FG motif with Ser or Ala residues prevents the transport from happening, and replacing Phe with aromatic Tyr or Trp residues reduces the binding strength. The amino acid that plays the central role in GLFG sequence is Leu. This amino acid is not present in the hydrophobic pocket of impβ; rather by protecting Phe from the solvent, it appears to affect the stability of the impβ–Nup bond (Patel and Rexach, 2008). It is hypothesized that some pockets on impβ have similar affinities to FG-Nups, even though affinities of other sites are different. Experiments illustrate a positive affinity gradient of Nups to impβ from the cytoplasmic to the nuclear side of

the NPC (Ben-Efraim and Gerace, 2001; Ben-Efraim et al., 2009). For instance, the affinity of impβ for Nup153 on the nuclear side is 20 times stronger than that of Nup358 on the cytoplasmic side (Fahrenkrog and Aebi, 2003). This characteristic makes the entering complex travel from low-affinity Nups to middle-affinity and finally to high-affinity ones unidirectionally, and this, as a result, enhances the transport efficiency (Ben-Efraim and Gerace, 2001). Recently, evidence confirming that the IBB domain modulates the avidity of impβ to Nups in the NPC channel was provided (Lott et al., 2010). Additionally, the bond between the RanGTP molecule and impβ in the nucleoplasm affects binding pockets on impβ, reducing the affinity of impβ to FG-Nups (Otsuka et al., 2008).

Other reports about distribution of transporter binding sites in the NPC suggest that attachment of transporters is localized around the channel entrance, though others reported a uniform distribution of transporter binding sites along the channel length (Fiserova and Goldberg, 2010; Kahms et al., 2009). In addition to known hydrophobic interactions between transporters and FG-Nups, more recent data suggest that electrostatic interaction between the transport receptors, which are highly negatively charged, and NPC compounds, which are positively charged, facilitates the selective transport across the NPC (Colwell et al., 2010).

2.3. Cargo complex dissociation

2.3.1. Nup50

Nup50 and its yeast homolog, Nup2p, are located on the nuclear basket (Fig. 6.2). These Nups are able to detach from the nuclear basket and shuttle between the nucleus and cytoplasm (Dilworth et al., 2001; Ogawa et al., 2010). Nup50 (Nup2p) is composed of an N-terminus domain, FG repeats, and a C-terminus domain (Fig. 6.10). Its N-terminus domain has affinity to the impα C-terminus and its NLS-binding site, and the FG repeat domain of this Nup, like other FG-Nups, has affinity to impβ (Fig. 6.10). The C-terminal domain contains a Ran-binding domain (RBD), having a weak affinity for RanGTP on the order of micromolar. Structural traits of these Nups give rise to a higher tendency of cargo–carrier complexes binding to them, more than any other nuclear basket Nup. These Nups increase the density of carriers in the proximity of their sites on the nuclear basket, thereby boost the probability of impacts needed for assembly/disassembly interactions. Hence, these Nups provide appropriate disassembly sites for imported complexes as well as assembly sites for exported cargos (Denning et al., 2002; Matsuura and Stewart, 2005; Matsuura et al., 2003; Swaminathan and Melchior, 2002).

Other Nups are also reported to mediate disassembly of imported cargos. For instance, Nup153, located on the nuclear ring, is capable of binding to RanGTP, via its Zinc Finger, and increasing RanGTP concentration in its

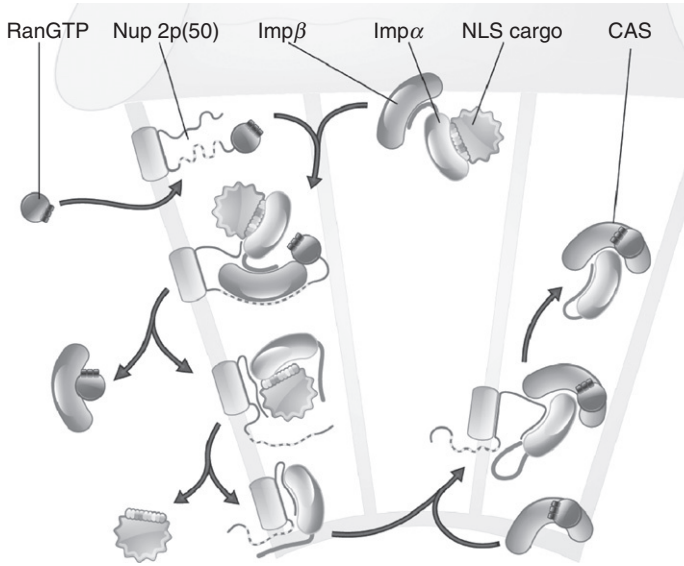

Figure 6.10 When the cargo complex enters the nucleocytoplasmic part and is bound to the Nup50 (Nup2p) FG repeat, the weak bond between RanGTP and Nup50 (Nup2p) breaks apart, releasing RanGTP to attach to impβ (Gilchrist and Rexach, 2003). Nup50 (Nup2p) primarily binds to the impα C-terminus with a high affinity. Following that, through a mechanism similar to that of IBB, it breaks the bond between NLS and impα, and finally, NLS detaches from impα, and Nup50 (Nup2p) substitutes it. Once the disassembly process takes place, impβ through binding to RanGTP easily recycles back to the cytoplasm, while impα requires another molecule termed CAS to exit (Cse1p). After Nup50 is bound to impα and NLS is detached from impα, CAS, which is attached to RanGTP, detaches Nup50 (Nup2p) from the impα C-terminus. Upon breakage of Nup50 (Nup2p), the IBB domain occupies the second binding site of Nup50 (Nup2p) to impα, which has lower affinity to impα, and is the NLS binding site. This is required to form the CAS–RanGTP–impα complex, and ultimately, Nup50 (Nup2p) detaches from the complex.

propinquity. As a result, Nup153 enhances impact probability of RanGTP and entering cargo complexes, and thereby it is highly involved in the cargo complex dissociation process (Schrader et al., 2008).

2.3.2. Ran

Ran is a 24-kDa member of the RAS family GTPase. Members of this family could attach either to GTP (guanosine-5′-triphosphate) or GDP (guanosine-5′-diphosphate). Ran has a core or G domain (guanine nucleotide-binding domain) including a p-loop, Switch I and II, and a 40-amino acid C-terminal extension (conserved across all Ran orthologs) consisting of a linker, an α-helix, and an acidic tail (DEDDDL) (Fig. 6.11).

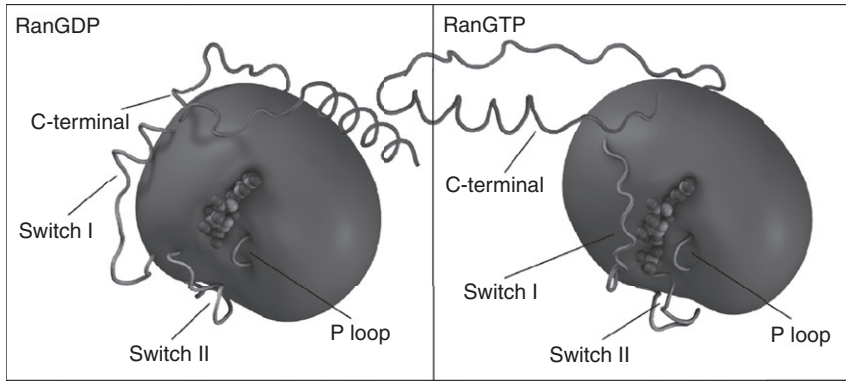

Figure 6.11 Ran: the overall structure of Ran remains somehow unchanged when it attaches to GTP or GDP, except in Switch I and II regions. In addition, the Ran C-terminus remodels upon its attachment to a nucleotide. The Ran C-terminus is disordered in the RanGTP complex (right) and is extended away from the complex core, whereas this extended portion touches the core in RanGDP at a few locations (left) (Fahrenkrog and Aebi, 2003; Lui and Huang, 2009; Rush et al., 1996; Scheffzek et al., 1995; Yudin and Fainzilber, 2009).

The overall structure of Ran remains almost unchanged when it attaches to GTP or GDP, except in Switch I and II regions, and in addition, the Ran C-terminus remodels upon its attachment to a nucleotide (Fig. 6.11). The Ran C-terminus is disordered in the RanGTP complex and is extended away from the complex core, whereas this extension touches the core in the RanGDP structure (Fig. 6.11; Fahrenkrog and Aebi, 2003; Lui and Huang, 2009; Neuwald et al., 2003; Rush et al., 1996; Scheffzek et al., 1995; Yudin and Fainzilber, 2009). This helical conformation obstructs the RanGDP-karyopherin attachment, while RanGTP is capable of binding to karyopherin members like impβ. Interestingly, it has been shown recently that kap95p and RanGDP are able to form a stable complex, which is capable of being unbound by impα. In kap95p–RanGDP complex, kap95p induces a conformational change in the RanGDP Switch I and II that makes conformation of these switches resemble those of RanGTP (Forwood et al., 2008).

2.3.3. Interactions that lead to the cargo complex disassembly

Disassembly of an entering complex could occur independent of Nup50 (Nup2p) presence. When a cargo complex enters the nucleoplasm, RanGTP can directly interact with impβ in absence of Nup50 (Nup2p). Such attachments release other factors in the complex of impβ via a conformational change. In other words, attachment of RanGTP to impβ induces a conformational change in impβ that unbinds its other partners

from it. Hence, the RanGTP–impβ bond is necessary to detach cargo–impα complex from impβ. The interaction between RanGTP and impβ occurs in three regions: at the first site, Switch II loop of RanGTP attaches to CRIME motif (CRM1, impβ, etc.), located in the impβ N-terminus on HEAT repeats 1–4 (Fig. 6.12). This bond exists in all members of the karyopherin family, as this site is similar in impβ, transportin, and Cse1p. At the second site, a basic part on the surface of the RanGTP G domain holds an electrostatic interaction with amino acids of the HEAT repeat 8 acidic loop in impβ (Fig. 6.12), thereby releases the IBB domain from the impβ HEAT repeat 8 region. Finally, at the third site, the Switch I loop and some parts of the amino acids of the RanGTP core interact with HEAT repeats and residues 12–15 on impβ. This local attachment is required to change the conformation, increase the helical pitch in the impβ, and eventually, detach the IBB domain.

It seems that Lys37 and Lys152 on RanGTP interact with the HEAT repeat 14 via electrostatic bonds. Mutations in Lys37 or Lys152 prevent the conformational change, thus obstruct the IBB detachment. Hydrogen bonds also have been observed in this site between Arg29, Arg154, and Arg156 on Ran and impβ amino acids. Also, evidence shows Phe35 and

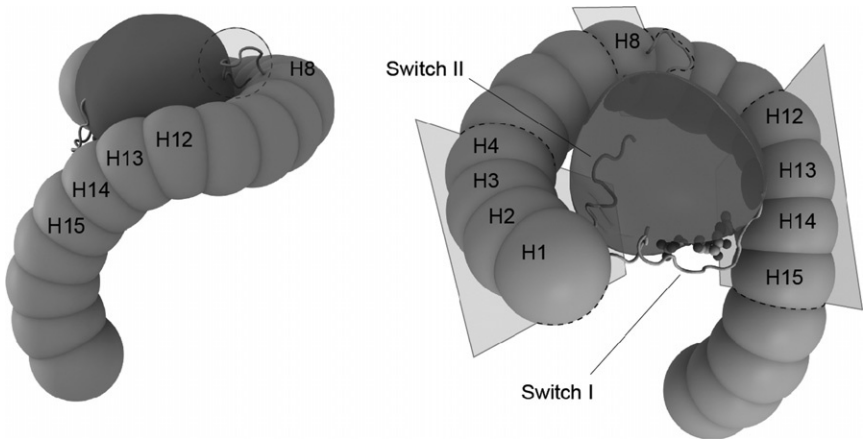

Figure 6.12 Importin-β–RanGTP complex: the interaction between RanGTP and impβ occurs in three regions: at the first site, the Switch II loop of RanGTP attaches to amino acids located in the impβ N-terminus on HEAT repeats 1–4. At the second site, the basic part on the surface of the RanGTP G domain holds an electrostatic interaction with amino acids of the HEAT repeat 8 acidic loop in impβ thereby, releasing the impβ IBB domain on the HEAT8 region. Finally, at the third site, the Switch I loop and some parts of the amino acids of RanGTP core interact with residues of HEAT repeats 12–15 on impβ. This local attachment is required to change the conformation, increase the helical pitch in the impβ, and eventually detach the IBB domain.

Phe157 amino acids hide together with impβ Phe613 and Leu563. Once impβ–RanGTP bond is formed, this complex is recycled back to the cytoplasm.

When the cargo–impα complex dissociates from impβ, the IBB domain is released and competes with the NLS for attachment to the binding site on impα through an autoinhibitory mechanism. It reduces the NLS–impα bond strength and eventually detaches the NLS. Following that, impα and impβ are exported to the cytoplasm separately from one another (Cook et al., 2007; Lee et al., 2005; Stewart, 2006, 2007; Xu and Massagué, 2004). Nevertheless, the NLS–impα bond has a high affinity of around 10 nM and is not easy to break apart, although Nup50 (Nup2p) helps alleviate this process (Fig. 6.10). In Nup50-dependent disassembly, when the imported cargo complex enters the nucleocytoplasmic part and is bound to a Nup FG repeat, the weak bond between RanGTP and Nup50 (Nup2p), which has a high dissociation rate (K_{off}), breaks apart, releasing RanGTP to let it attach to impβ (Fig. 6.10) (Gilchrist and Rexach, 2003). This newly formed complex detaches from the entering complex and is exported to the cytoplasm.

Kinetic studies indicate that Nup50 (Nup2p) in addition to increasing RanGTP and cellular apoptosis susceptibility (CAS) protein concentration in the nuclear basket causes active detachment of the NLS from impα (Fig. 6.10). Nup50 (Nup2p) primarily binds to the impα C-terminus with a high affinity. Following that, using a mechanism similar to that of the IBB, it breaks the bond between the NLS and impα, and finally, NLS detaches from impα and Nup50 (Nup2p) substitutes it (Fig. 6.10). Nup50 (Nup2p)–impα affinity at this binding site is higher than the affinity at the impα. Moreover, these Nups bind to impα with higher affinities than do NLSs, through their electrostatic, hydrophobic, and hydrogen bonds. The Nup2p N-terminus attaches to kap60p without IBB with an affinity of about 2.4 nM, while the NLS attaches to the kap60p without IBB domain with an affinity of approximately 10–30 nM, resulting in kap60p binding more firmly to Nup2p than it is doing to the NLS. Presence of Nup50 increases the NLS dissociation rate from impα by an order of magnitude relative to the spontaneous NLS dissociation rate in the absence of Nup50. The Nup50-dependent pathway is crucial for the detachment of the bipartite NLS as the IBB is not able to conduct this process on its own.

In general, Nup50 facilitates the NLS detachment in two ways: Nup50 is able to interact with imported impα–impβ–NLS complex and once the NLS is released, RanGTP detaches impβ from the complex. At the end, RanGTP–CAS complex releases Nup50 and recycles impα back to the cytoplasm. The alternate way to expedite the NLS detachment reaction is Nup50 coming into play immediately after impβ is detached by RanGTP (Matsuura and Stewart, 2005; Matsuura et al., 2003; Moore, 2003; Sun et al., 2008).

Since Nup50 structure is capable of binding to impα, impβ, and RanGTP, and it is a dynamic protein, a model based upon the presence of Nup50 as an entering complex has also been suggested. In this model, Nup50 is able to bind to both impα (attached to the NLS) and impβ in the cytoplasm and thereby increases assembly of the entering quadruple complex. This complex passes across the central channel and enters the nuclear basket, where binding of RanGTP to Nup50 initiates a complex disassembly process, and eventually, two triple complexes, i.e. Nup50–RanGTP–impβ and impα–CAS–RanGTP, are formed and transported back to the cytoplasm (Moore, 2003; Swaminathan and Melchior, 2002). It has been discovered recently that two Nup50 isoforms, called Npap60s and Npap60l, regulate nuclear import of proteins. In other words, alterations in the expression level of these two Nups, which act in opposite ways to release the NLS in the nuclear basket, control efficiency of the nuclear import. This phenomenon confirms the role of Nups in regulating the import efficiency (Ogawa et al., 2010).

2.4. Karyopherin recycling

Once the disassembly process takes place impβ easily recycles back to the cytoplasm via binding to RanGTP; however, impα requires a CAS molecule to exit the nucleus (Fig. 6.10). After Nup50 is bound to impα and the NLS is detached from impα, CAS (Cse1p), which is attached to RanGTP, detaches Nup50 (Nup2p) from the impα C-terminus (Fig. 6.10). Upon breakage of Nup50 (Nup2p) strong bonds, the IBB domain occupies the second binding site of Nup50 (Nup2p) to impα, which has lower affinity to impα and in fact is the NLS binding site. This is required to form the CAS–RanGTP–impα complex (Fig. 6.13), and ultimately, Nup50 (Nup2p) detaches from this complex. Nup50 (Nup2p) attachment to impα, its detachment by CAS (Cse1p), and the strong binding of CAS to impα guarantee that impα returns to the cytoplasm only after cargo is already released (Matsuura and Stewart, 2004, 2005).

2.4.1. CAS (Cse1p)

Cse1p is made up of 20 HEAT repeats that are stacked up. HEAT repeat motifs of CAS (Cse1p) are composed of 40 amino acids. These HEAT repeats comprise two antiparallel α-helices called A and B, shaping up the internal and external faces of Cse1p, respectively. A and B helices are connected by a tiny loop in all HEAT repeats except in the HEAT repeat 19 helices, where they are connected by a long loop. Also, in HEAT repeat 8 helices, an intrarepeat connection replaces the tiny connection loop. Not only does RanGTP cause dissociation of importins and their partners in the nucleus, but also it mediates the CAS attachment to impα. Therefore, these

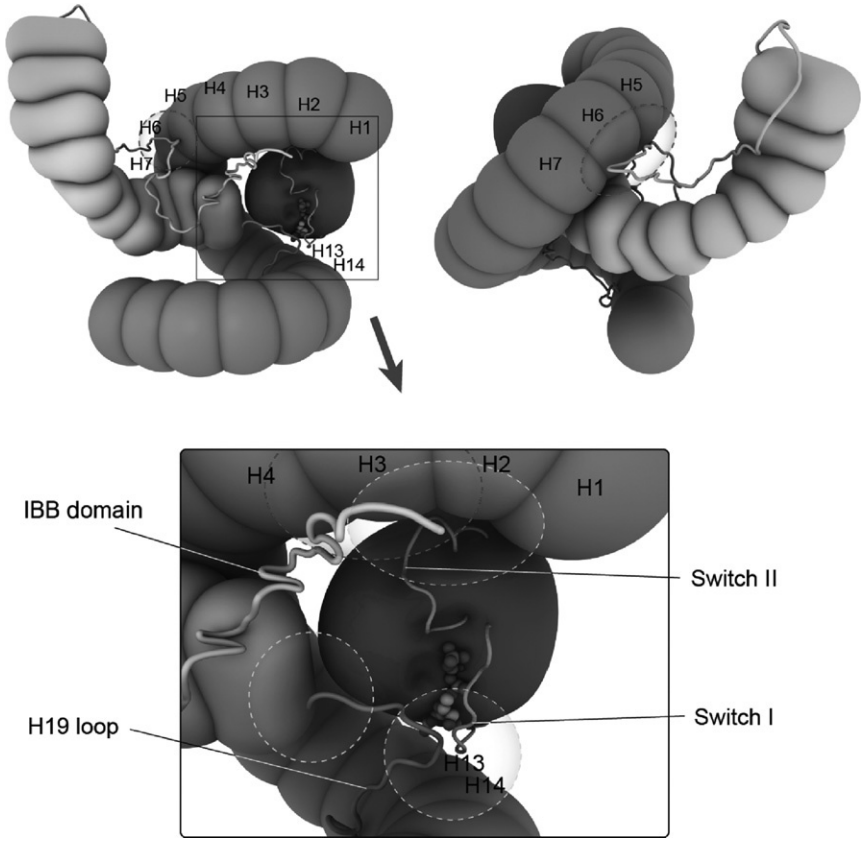

Figure 6.13 Cse1p–kap60p–RanGTP complex: when kap60p is absent RanGTP only possesses a single binding site on Cse1p (its HEAT 1–3) through Arg76 and Asp77 on the Switch II loop. In presence of kap60p, Cse1p distorts and RanGTP binds to the second binding site on Cse1p, i.e. HEAT 13–14 and the long loop of HEAT 19 via its Lys37 on the Switch I loop and Lys152, which strengthens the RanGTP–Cse1p bond. Hence, in Cse1p, Ran is located around the center, surrounded from two sides by arch-like structures of HEAT repeats 1–3 and HEAT repeats 13–14, and it interacts with HEAT19. Other than being a mere binding site, HEAT19 acts to prevent detachment of guanine nucleotide. Upon the binding of kap60p to the Cse1p–RanGTP complex the IBB N-terminus interacts with Cse1p HEAT 2–4 and so does a tiny portion of the IBB middle part with the HEAT 5–7 external surface. This way, IBB is engaged in forming the kap60p–RanGTP–Cse1p triple complex. Moreover, the only part of Cse1p that is forming bonds with both RanGTP and kap60p in the Cse1p–RanGTP complex is HEAT 19.

processes orchestrate an on-time association/dissociation via regulating the affinity of impβ and CAS to their partners (Matsuura and Stewart, 2004; Zachariae and Grubmüller, 2006).

2.4.2. Interactions forming a recycling complex

When kap60p is absent RanGTP possesses a single binding site on Cse1p (its HEAT 1–3) through Arg76 and Asp77 on the Switch II loop. In presence of kap60p, Cse1p distorts and RanGTP binds to the second binding site on Cse1p, i.e. HEAT repeats 13–14 and the long loop of the HEAT repeat 19, via its Lys37 on the Switch I loop and Lys152, and this strengthens the RanGTP–Cse1p bond (Fig. 6.13). Hence, in Cse1p, Ran is located around the center surrounded from two sides by arch-like structures of HEAT repeats 1–3 and HEAT repeats 13–14, and it interacts with the HEAT repeat 19 (Fig. 6.13). In addition to being a binding site, HEAT repeat 19 acts to prevent detachment of guanine nucleotide. Upon the binding of kap60p to the Cse1p–RanGTP complex, the IBB N-terminus interacts with Cse1p HEAT repeats 2–4 and so does a tiny portion of the IBB central region with the HEAT repeats 5–7 external surface, this way IBB is engaged in forming the kap60p–RanGTP–Cse1p triple complex (Fig. 6.13). Moreover, the kap60p C-terminus is involved in an interaction with Cse1p and RanGTP, and the only part of Cse1p engaged in bonds with both RanGTP and kap60p in the Cse1p–RanGTP complex is HEAT repeat 19 (Fig. 6.13). RanGTP amino acids Arg95, Lys99, Lus130, and Lys134 are essential to its interaction with kap60p (Matsuura and Stewart, 2004). When Ran is absent because of ineffectiveness of collisions, no bonds form during the CAS interaction with impα.

CAS has a closed conformation in its free state and a decreased helical pitch as opposed to impβ (Fig. 6.14). CAS takes on an open conformation when it connects to a cargo, and this conformational change (open-to-closed) is caused by bonds forming between CAS N-terminus residues and a region close to its C-terminus and central region (Fig. 6.14; Conti et al., 2006; Stewart, 2006). Molecular dynamics (MD) simulations indicate that the Cse1 structure collapses spontaneously via electrostatic interactions during extremely short-time scales (10 ns), forming a close cytoplasmic structure. Moreover, MD studies conducted on mutations of these electrostatic interactions revealed their significance in triggering a conformational change from open to closed (Zachariae and Grubmüller, 2006). During the bonding process, the HEAT repeat 14 moves along relative to HEAT repeats 1–3, forming a cluster of acidic residues of Glu652, Asp653, and Glu656 on HEAT repeat 14 with a charged chain of Lys21, Lys30, Arg25, and Arg28 on HEAT repeats 1–2. Moreover, HEAT repeats 2–3 loop amino acids (i.e., Glu72 and Asp71) hold an electrostatic interaction with HEAT repeats 15–16 amino acids (i.e., Arg728 and Lys695, Lys733). Simulations suggest CAS conformational closing occurs as a result of formation of an interaction between HEAT repeats 1 and 3 and HEAT repeats 14 and 17 residues after they move toward each other (Fig. 6.14; Zachariae and Grubmüller, 2006). Altogether with some other polar interactions, these bonds hide the Ran-binding site considerably. The HEAT repeat 19

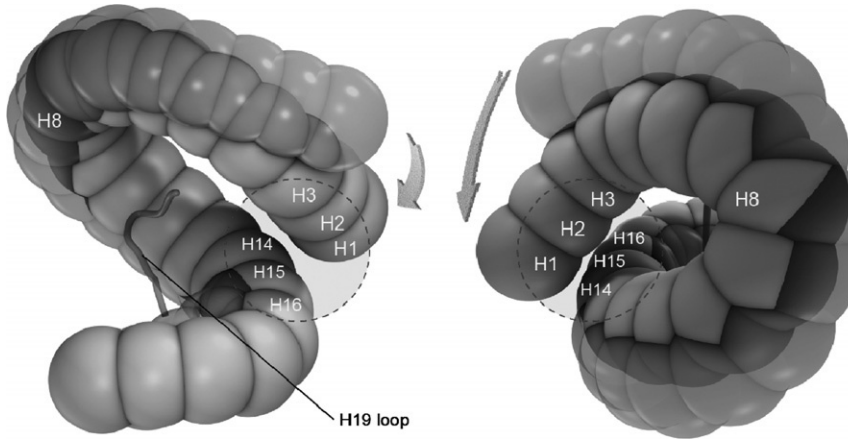

Figure 6.14 CAS (Cse1p) conformation: CAS has a closed conformation in free state and a decreased helical pitch as opposed to impβ. It (the transparent one) opens up its conformation when it connects to a cargo. The conformational change (open-to-closed) is caused by bonds between CAS N-terminus residues and a region close to the C-terminus and the center. During the bonding process, HEAT repeat 14 moves along relative to HEAT repeats 1–3, forming a cluster of acidic residues on HEAT repeat 14 with a charged chain on HEAT repeats 1–2. Moreover, HEAT repeats 2–3 loop amino acids hold an electrostatic interaction with HEAT repeats 15–16 amino acids. HEAT repeat 19 loop, which mediates the attachment of impα and RanGTP to CAS while forming free CAS, changes its conformation as a result of its clash with the N-terminus so that protease could affect it and cut it off, and hence, free CAS lacks HEAT repeat 19 loop. In closed-to-open conformational change, the most important rearrangement region is located on HEAT repeat 8. This HEAT repeat is in fact a hinge region, and having a conserved insertion, it acts like a switch. It causes conformational opening by allowing the interaction between RanGTP, impα, and CAS to occur.

loop, which mediates the attachment of impα and RanGTP to CAS while forming free CAS, changes its conformation as a result of its clash with the N-terminus so that protease could affect it and cut it off, and hence, the free CAS lacks the HEAT repeat 19 loop (Fig. 6.14). This conformational change is a regulatory mechanism to prevent impα from binding to CAS at a wrong time and keeps CAS and impα bound to each other in the nucleus. In the closed-to-open conformational change, the most important rearrangement region is located on HEAT repeat 8 (Fig. 6.14). This HEAT repeat is in fact a hinge region and having a conserved insertion, it acts like a switch. It causes a conformational opening by allowing the interaction between RanGTP, impα, and CAS to occur. In the closed conformation, the impα binding site is rearranged and RanGTP is closed. During the closed-to-open conformational change a movement around the hinge zone (i.e., the HEAT repeat 8) exposes some of the binding sites of RanGTP on HEAT repeat 19 of CAS to an interaction. This enables impα to gain access

to some binding sites, especially the CAS N-terminus. After the impα–RanGTP–CAS complex forms, it is transported to the cytoplasm. In this pathway, the complex is propelled in channel by hydrophobic interactions of FG-Nups with CAS. Once it reaches the cytoplasm, the complex attaches to RanBP1 or RanBP2, RanGAP (these proteins will be explained in Section 2.5), catalyzes the hydrolysis of RanGTP, and in the end, the complex disassembles, separating impα and CAS from each other and preparing them for the next transport cycle (Zachariae and Grubmüller, 2006). In addition to these models, single molecule fluorescence resonance energy transfer (FRET) studies showed that impα–cargo complex disassembly takes place in the NPC channel in presence of CAS and RanGTP, and afterward, most of the dissociated molecules penetrate the nucleus while nondissociated complexes return to the cytoplasm (Sun et al., 2008).

2.5. Ran cycle

Ran and its regulators are key components of the nucleocytoplasmic pathway and most of other vital pathways of the cell, such as RNA synthesis, mitosis, etc. This small molecule exists in both import and export pathways and indeed, it determines the transport direction. In the nucleus, RanGTP substitutes RanGDP and travels back to the cytoplasm by attaching to the export complex. In the cytoplasm, however, RanGTP is hydrolyzed, providing RanGDP again for further use. The rate of Ran export from the nucleus is extraordinarily high (10^5 copies per second) (Batrakou et al., 2009; Lui and Huang, 2009; Yasuhara et al., 2009; Yudin and Fainzilber, 2009). In fact, RanGTP (active form of Ran) is a nucleocytoplasmic pathway controller and its nuclear concentration is higher than that of RanGDP (inactive form of Ran), as opposed to its cytoplasmic concentration. This concentration gradient provides the energy required to regulate and direct transport (GoÈrlich et al., 2003). *In vitro* studies confirmed that reversing the RanGTP concentration gradient leads to a reversal in the transport direction. Thus, it could be stated that the RanGTP/RanGDP gradient controls the transport direction and it provides a driving force for transport across the NPC (GoÈrlich et al., 2003).

RanGAP (Ran GTPase activating protein) and RanGEF (Ran guanine nucleotide exchange factor) mediate the formation of a vital switch between RanGTP and RanGDP. In the cytoplasm, RanGAP catalyzes hydrolysis of GTP, which exists in RanGTP along with RanBP1 or RanBP2 factors and finally, produces RanGDP via imposing conformational changes to Ran (Figs. 6.15 and 6.16). In the nucleus, RanGEF with intervention of RanBP3 separates GDP nucleotide from Ran. It stabilizes the Ran and eventually replaces GDP with GTP and thereby causes RanGTP to accumulate in the nucleus via RanGEF (Fig. 6.19). There are approximately 3×10^5 RanGAP copies and almost the same number of Rcc1 (regulator of

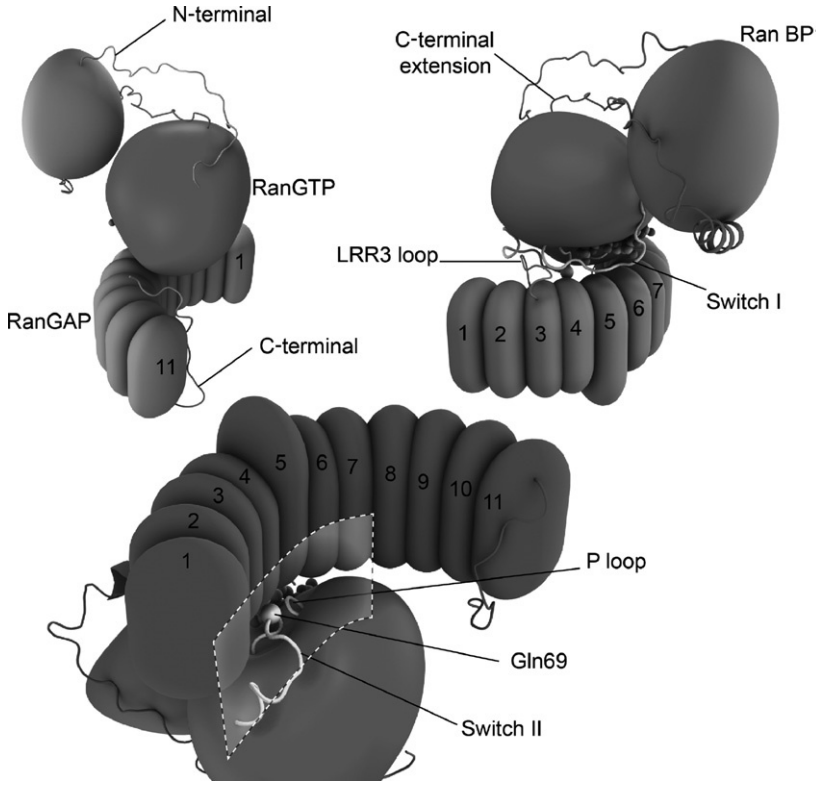

Figure 6.15 RanBP1–RanGTP–RanGAP complex: in this complex, RanGAP and RanBP1 bind to opposite sides of Ran resulting in no interaction between these two agents, plus RanGAP is located on the edge of RanGTP. Therefore, RanBP1 excites RanGAP indirectly by affecting Ran. The Ran acidic C-terminal extension wraps around the basic RanBD patch. The acidic hand on the RanBD N-terminus stretches across to attach to the basic patch on Ran. Hence, the body of the RanBD is held closely against the switch I region and against other residues in the C-terminal half of the Ran protein (Bischoff and Gorlich, 1997; Lounsbury and Macara, 1997; Saric et al., 2007; Seewald et al., 2003). RanGTP holds electrostatic interactions with residues of seven Leu-rich repeats (LRR) of RanGAP. A grown loop in the third LRR acts as a footrest, stabilizing the Switch II region of Ran. RanGAP stimulates RanGTPase activity by stabilizing the Switch II and correcting orientation of catalytic glutamine of Ran. In fact, when RanGAP touches the Ran Switch II and p-loop, it induces an alteration in orientation of one of their amino acids, i.e. Gln69 (Cook et al., 2007; Madrid and Weis, 2006; Seewald et al., 2002).

chromosome condensation1: mammalian RanGEF) copies in a single cell, hence, the hydrolysis capacity of GTP is balanced with its exchange capacity (Bos et al., 2007; Lui and Huang, 2009; Macara, 2001; Nishimoto, 2000; Rush et al., 1996).

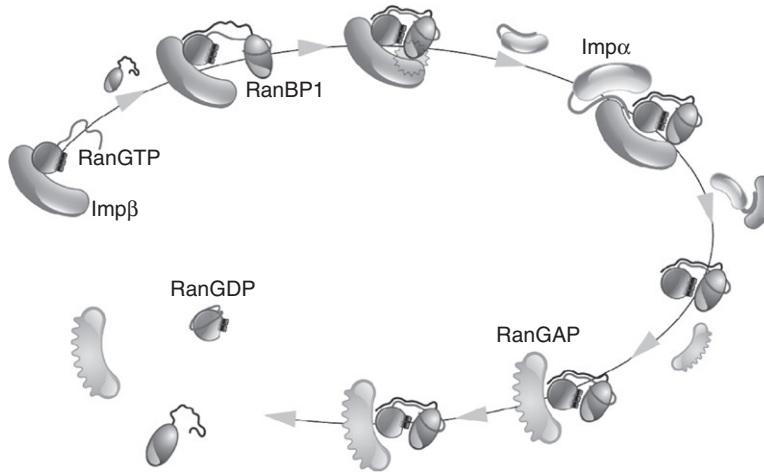

Figure 6.16 In the triple RanBD–RanGTP–impβ complex, the extension of the Ran C-terminus in the karyopherin–RanGTP complex suggests that RanBD is able to access the Ran C-terminus. The Ran acidic C-terminal extension with the DEDDDL motif wraps around the basic RanBD patch. Upon the binding of RanBD to the impβ–RanGTP complex, clash of RanBD to impβ (sterical hindrance) facilitates impβ detachment, and it eventually dissociates in the presence of impα. Afterward, the acidic hand on the RanBD N-terminus stretches across to attach to the basic patch on Ran. This interaction obstructs rebinding of impβ to the RanBD–RanGTP complex and is essential for the detachment to occur. Hence, RanBD is held closely against the Switch I region and against other residues in the C-terminal portion of the Ran protein. At this time, RanGAP comes into play and provokes RanGTP hydrolysis by binding to the RanBD–RanGTP complex. The RanBD connection to the Ran–importin complex pulls the Ran C-terminus aside, facilitating the RanGAP attachment to RanGTP (Bischoff and Gorlich, 1997; Lounsbury and Macara, 1997; Petersen et al., 2000; Saric et al., 2007; Seewald et al., 2002, 2003). So, is RanGAP able to interact with RanGTP, forming a quadruple complex along with RanBD and impβ, or is impβ released first and then RanGAP fulfills its job? (Melchior and Gerace, 1998).

2.5.1. Ran's GTP hydrolysis

The hydrolysis rate of RanGTP with Ran is low ($k_{cat} = 1.8 \times 10^{-5}\ s^{-1}$). However, RanGAP increases this rate up to $k_{cat} = 2–10\ s^{-1}$. Hence, RanGAP is able to increase the RanGTP hydrolysis rate by as much as 10^5-fold up to 5 RanGTP/Sec. RanGAP is composed of a symmetric structure of 11 Leu-rich repeats (LRR) (Fig. 6.15). This crescent-like structure is a compound of a number of helices and hairpins. RanGAPs of different species share the same catalytic N-terminal domain. Further, the RanGAP C-terminus, which has 230 amino acids, is conserved among some species of eukaryotes (Fig. 6.15). In budding yeast, the RanGAP NLS and NESs facilitate its transport into and out of the nucleus. In vertebrates, SUMO modification (a polypeptide dependent on ubiquitin)

occurs on the RanGAP C-terminus covalently. This increases the tendency of RanGAP to interact with RanBP2/Nup358 which is a component of cytoplasmic fibrils (Fig. 6.2), and this finally expedites the hydrolysis rate. In fact, Nup358 modifies RanGAP through the activity of its Sumo E3 ligase, while RanGAP diffuses in the yeast cytoplasm, since it lacks a RanBP2/Nup358 homolog (Seewald et al., 2002).

Recent works also indicate that Nup358–RanGAP complex plays a crucial role in impα/β-dependent nuclear import and disassembly of the export complex. Generally speaking, two pathways are proposed in this regard. The first one suggests disassembly of the RanGTP–impβ complex, which has entered the cytoplasm from the nucleus, and the formation of a new impβ–impα–NLS imported complex by mediation of soluble RanGAP and RanBP1 (Fig. 6.19). The second pathway is based on the interaction of recycled-to-the-cytoplasm RanGTP–impβ with Nup358/RanBP2, which is in contact with RanGAP. Following this event, RanGTP is hydrolyzed and a new entering complex is formed through the attachment of impα and NLS (Fig. 6.19; Hutten et al., 2008; Nishimoto, 2000).

In fact, RanGTP should be protected against GTP hydrolysis caused by RanGAP and against the exchange caused by Rcc1, and this task is performed by karyopherins. Basically, karyopherins when bound to RanGTP block the access of RanGAP to the RanGTP Switch II (Figs. 6.15 and 6.16), and this prevents RanGAP from binding to RanGTP while it is carrying a karyopherin. Instead, RanBP2 or RanBP1 is able to bind to Ran in this case. In vertebrates, interactions of importin with Nup358/RanBP2 increase off rate of RanGTP from importins. RanBP2 has four RanBDs (Ran-binding domains), and structural investigations of RanGTP while bound to the first and second RanBD of RanBP2 showed RanBD has a Pleckstrin Homology fold domain (PH domain), which stretches up to the Ran Switch I and its N-terminus loops around RanGTP. Additionally, the RanGTP C-terminus wraps around RanBD and these factors cooperate to hydrolyze RanGTP to RanGDP in the cytoplasm (Geyer et al., 2005; Petersen et al., 2000).

RanBP1 is a 23-kDa protein factor equipped with RanBD (Fig. 6.15). RanBD binds to RanGTP with a high affinity, increasing the activity of RanGAP to hydrolyze GTP by 10-fold (Madrid and Weis, 2006; Seewald et al., 2003). In the triple RanBD–RanGTP–impβ complex, the RanBD binding site on RanGTP does not overlap with the impβ binding site on RanGTP. Also, the extension of the Ran C-terminus in the karyopherin–RanGTP complex suggests that RanBD is able to access the Ran C-terminus. The Ran acidic C-terminal extension with the DEDDDL motif wraps around the basic RanBD patch (Fig. 6.16). Upon the binding of RanBD to the impβ–RanGTP complex, collisions of RanBD to impβ (sterical hindrance) facilitate impβ detachment and it eventually dissociates in the presence of impα (Fig. 6.16). Afterward, the acidic hand on the N-terminus of

RanBD stretches across to attach to the basic patch on Ran. This interaction obstructs rebinding of impβ to the RanBD–RanGTP complex and is essential for the detachment to occur (Fig. 6.16). Hence, RanBD is held closely against the Switch I region and against other residues in the C-terminal portion of the Ran protein. At this time, RanGAP comes into play and excites RanGTP hydrolysis by binding to the RanBD–RanGTP complex (Fig. 6.16). The RanBD connection to the Ran–importin complex pulls the C-terminus of Ran aside, facilitating the RanGAP attachment to RanGTP (Bischoff and Gorlich, 1997; Lounsbury and Macara, 1997; Petersen et al., 2000; Saric et al., 2007; Seewald et al., 2002, 2003). So, is RanGAP able to interact with RanGTP, forming a quadruple complex along with RanBD and impβ, or is impβ released first and followed by that RanGAP fulfills its task? This question calls for further investigation through this intricate biochemical machine (Melchior and Gerace, 1998).

In the CAS–impα–RanGTP complex exiting the nucleus, the RanBD–RanGTP connection causes the detachment of CAS from RanGTP and the binding of RanGAP to the complex (Bischoff and Gorlich, 1997). In the RanBP1–RanGTP–RanGAP complex (Fig. 6.15), RanGAP and RanBP1 bind to opposite sides of Ran which results in no interaction between these two agents; moreover, RanGAP is located on the edge of RanGTP. Therefore, RanBP1 excites RanGAP indirectly by affecting Ran. RanGTP holds electrostatic interactions with residues of seven Leu-rich repeats (LRR) of RanGAP. A grown loop in the third LRR acts as a footrest, stabilizing the Switch II region of Ran (Fig. 6.15). In other small GTPases, GAP-assisted GTP hydrolysis is mediated by an Arg residue of GAP, while the Arg finger is not observed in the RanGTP–RanBP1–RanGAP complex. RanGAP stimulates RanGTPase activity through stabilizing the Switch II and correcting orientation of catalytic glutamine of Ran. In fact, when RanGAP touches the Ran Switch II and p-loop, it induces an alteration in orientation of one of their amino acids, i.e. Gln69. A mutation in Gln69 reduces the RanGAP activity thus, declines the GTP hydrolysis rate in RanGTP (Fig. 6.15) (Cook et al., 2007; Madrid and Weis, 2006; Seewald et al., 2002). It is known that in the nucleus, Tyr39 on Ran holds Gln69 of Ran, protecting this amino acid from water molecule invasion. Conversely, in the cytoplasm, Asn133 of RanGAP interacts with Gln69 of Ran thereby, enables the correct position and water invasion via displacing Tyr39 (Brucker et al., 2010).

2.5.2. NTF2

Nuclear transport factor2 (NTF2) is a dedicated carrier for RanGDP shuttling back and forth from the cytoplasm to the nucleus. NTF2 is a barrel-like homodimer that is conserved among all eukaryotes and has two hydrophobic pockets. RanGDP can attach to these pockets with its Switch I and Switch II, in a large contact interface. During its transport through the

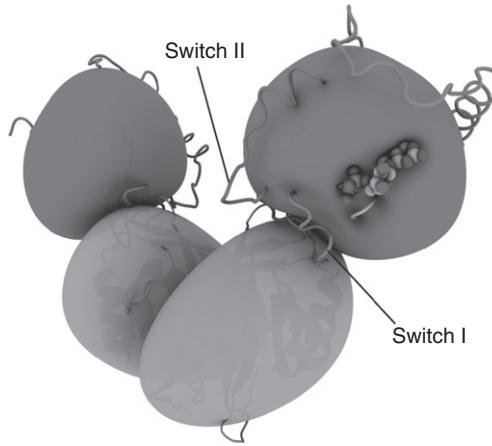

Figure 6.17 NTF2 (lighter color) is a homodimer molecule that has two hydrophobic pockets. RanGDP can attach to these pockets with its Switch I and Switch II, in a large contact interface. During its transport through the NPC, NTF2 interacts with FG repeats via its hydrophobic end. These FG repeats bind to two symmetric hydrophobic binding sites on the interface of the two dimmers (Madrid and Weis, 2006).

NPC, NTF2 interacts with FG repeats from its hydrophobic end. FG repeats bind to two symmetric hydrophobic binding sites on the interface of the two dimmers (Madrid and Weis, 2006). However, simulations identify six adjacent binding spots for FG repeats on NTF2 import and export complexes (Fig. 6.17) (Isgro and Schulten, 2007). Rcc1 releases RanGDP from NTF2 in the nucleus and consequently, NTF2 returns to the cytoplasm. The comparison between the RanGDP–NTF2 and Ran–Rcc1 complexes shows that Ran cannot bind to NTF2 and Rcc1 simultaneously. This is consistent with the fact that NTF2 obstructs dissociation of Ran and GDP by Rcc1. Hence, it suggests that RanGDP should detach from NTF2 before the nucleotide exchange occurs (Fig. 6.17) (Chumakov and Prasolov, 2010; Madrid and Weis, 2006; Renault et al., 2001).

2.5.3. Ran's nucleotide exchange

RanGEF (see Fig. 6.18) has a donut-like structure composed of seven bladed propellers at the periphery and a hole near its center. It possesses an acidic residue that interacts with one of Lys' on Ran. This structure is attached at one end to Ran and at the other to chromatin. It is speculated that RanGEF is bound to H2A and H2B of nucleosomes with a high affinity. However, it is also likely that RanGEF is rather bound to an internucleosome-exposed zone. Although RanBP3 is necessary to activate

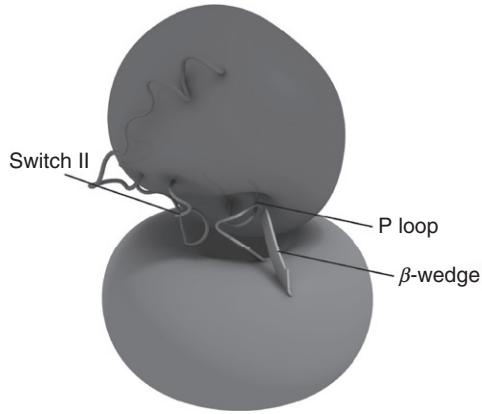

Figure 6.18 Rcc1 (lighter) has a donut-like structure that induces disorder in the acidic Ran C-terminus. Therefore, it takes part in the detachment of GDP from RanGDP. Rcc1 inserts its β-hairpin, which acts as a β-wedge, into the Switch II and Ran p-loop, which is the nucleotide-binding site. Therefore, causes the GDP separation; a conformational change different from that occurs to Ran during its attachment to GDP and GTP.

RanGEF, it is observed that histones could even double the activity of RanGEF. Attachment of Rcc1 to chromatin is necessary for NCT and cell mitosis to occur (Chumakov and Prasolov, 2010; Fuller, 2010; Madrid and Weis, 2006; Yudin and Fainzilber, 2009). Metazoan and yeast RanGEFs are called Rcc1 and prp20, respectively. Rcc1 is a 45-kDa structure that induces disorder in the Ran acidic C-terminus. Therefore, it takes part in the detachment of GDP from RanGDP. Rcc1 inserts its β-hairpin, located on its third blade propeller which acts as a β-wedge, into the Switch II and Ran p-loop, which is the nucleotide-binding site and in so doing causes the GDP separation; a conformational change different from that occurs to Ran during its attachment to GDP and GTP (Fig. 6.18). Briefly, Rcc1 destabilizes the Ran–GDP complex by inducing a conformational change in Switch II, p-loop, and the RanGDP C-terminus. After GDP is separated, since the concentration of GTP is higher than that of GDP and Rcc1 expression is increased, GDP is replaced with GTP readily via Rcc1 mediation. This interaction progresses very slowly in the absence of Rcc1 (Bos et al., 2007; Lui and Huang, 2009; Renault et al., 2001; Vetter and Wittinghofer, 2001).

Apparently, the attachment of RanGEF to Histones enhances RanGEF activity up to twice as much. This bond induces a mild conformational change in the Rcc1 binding site to His. This conformational change propagates toward the nucleotide-binding site of Ran through the β-wedge of Rcc1, and therefore, Rcc1 attachment to the nucleosome increases the guanine nucleotide exchange. Further, a model has been proposed

suggesting a direct bond between Ran and both Rcc1 and nucleosome (England et al., 2010).

The effect of Rcc1 is to such a degree that guanine nucleotide exchange reaction occurs 10^5 times faster in the presence of Rcc1. Moreover, the spontaneous dissociation half-life of GDP from Ran is around 2 h at 25 °C, which could be further shortened by Rcc1 facilitation (Klebe et al., 1995). Rcc1 has an NLS on its N-terminus and rides on importins to enter the nucleus. An accompanying importin is, however, not a requirement for entrance, and substitute pathways potentially exist.

Recently, a model has been suggested in which impβ works in Ran as the exchanging factor of GTP to GDP. In this model, the RanGDP–RanBP1–impβ–impα–NLS complex forms in the cytoplasm and NTF2 facilitates its passage across the channel. Also, presence of NTF2 protects the complex against GDP nucleotide exchange. Upon passing across the channel, NTF2 is separated by an unknown factor and GDP is replaced with GTP. In fact, Ran affinity to GTP when bound to impβ is more than that to GDP, and this is the key factor that makes the nucleotide exchange possible. Once RanGTP is formed and a strong interaction with impβ occurs, impα and NLS are detached from the complex (Lonhienne et al., 2009).

3. Nucleocytoplasmic Transport Pathway

The main NCT pathways are shown in Fig. 6.19. Nuclear pore complexes mediate bidirectional transport of various macromolecules. At first, in the cytoplasm, the NLS-carrying cargo through interaction with impα binds to the impα/impβ complex to form an import complex, which then docks the cytoplasmic filaments. This complex passes across the NPC toward the nucleus via weak interactions with FG-Nups. Inside the nucleus, the imported complex attaches to the nuclear basket Nups such as Nup50 (Nup2). After binding of import complex to Nup50 occurs, RanGTP, which is in high concentration near the nuclear basket, dissociates impβ from the cargo complex and the new RanGTP–impβ complex is recycled back to the cytoplasm. Subsequently, cargo detaches from impα via a connection of Nup50 to impα. Then, impα in the presence of a CAS molecule, in a complex with RanGTP, is detached from Nup50, and finally is exported back to the cytoplasm, attaching to the CAS–RanGTP complex. In the cytoplasm, the impα–CAS–RanGTP and RanGTP–impβ complexes should be disassembled into their components.

Disassembly of the RanGTP–impβ complex may occur by intervention of soluble RanGAP and RanBP1 (Fig. 6.19), or Nup358/RanBP2, which is in contact with RanGAP; RanGTP is hydrolyzed later on (Fig. 6.19; Hutten et al., 2008; Nishimoto, 2000). In fact, once attachment of this

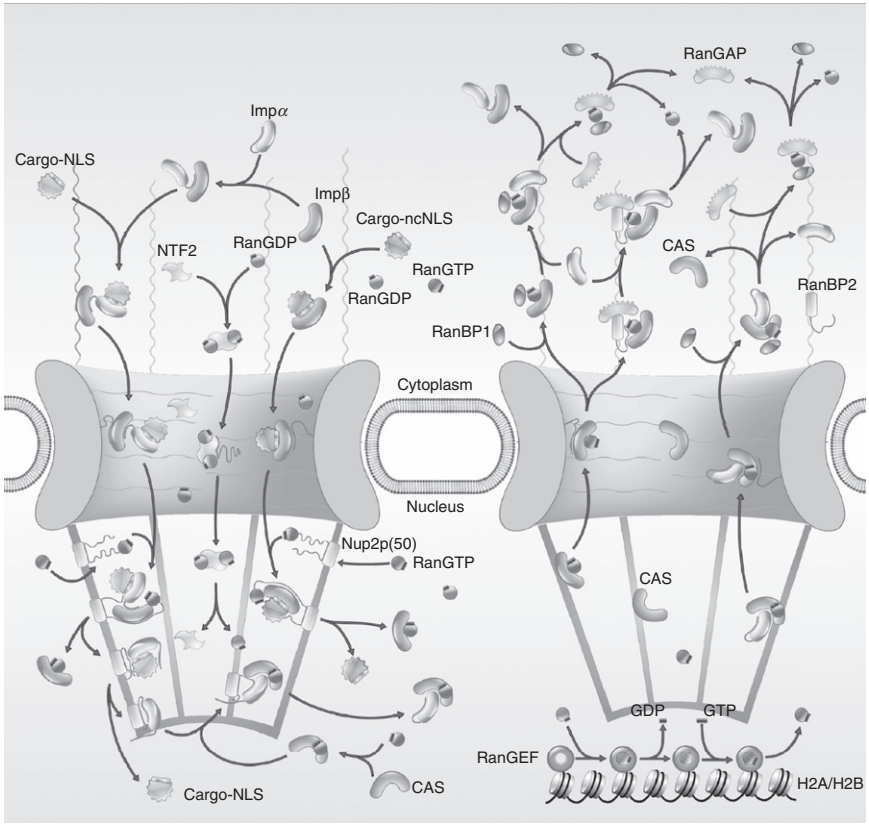

Figure 6.19 **The nucleocytoplasmic transport (NCT) pathways.**

complex to RanBP1 takes place, in the presence of impα, impβ is released. RanGAP through binding to RanGTP excites the GTP-to-GDP hydrolysis. Ultimately, the RanGAP–RanGTP–RanBD complex dissociates upon formation of RanGDP. However, the impβ–RanGTP complex through binding to RanBP2/Nup358, which is connected to RanGAP, forms a quadruple complex. Afterward, the presence of impα helps destabilize the complex–impβ interaction and enables RanGAP to bind to RanGTP and provoke its hydrolysis. Impβ and impα detach from the complex when the RanGTP hydrolysis is over. Indeed, it is not known whether RanGAP comes into play immediately after the complex enters the cytoplasm and causes its disassembly via provoking RanGTP hydrolysis, or impβ detaches from the complex prior to the action of RanGAP.

While the CAS–impα–RanGTP complex is exiting the nucleus, the RanBD–RanGTP connection causes the detachment of CAS from RanGTP, and eventually, the hydrolysis of RanGTP is catalyzed by the

binding of RanGAP to the complex (Bischoff and Gorlich, 1997). However, some researchers believe RanBP1 and RanGAP together attach to the CAS–impα–RanGTP complex, and after the hydrolysis of GTP, because CAS does not have a high affinity to RanGDP, the complex is disassembled (Kutay et al., 1997).

Cargos carrying an NES attach to the exportin–RanGTP complexes, such as Crm1–RanGTP and return to the cytoplasm. The abovementioned hydrolysis process gives rise to RanGTP conversion into RanGDP and releases the cargo. However, NTF2 is in charge of carrying RanGDP from the cytoplasm to the nucleus. In the nucleus, NTF2 detaches from RanGDP, and RanGEF catalyzes the replacement of GDP by GTP, preparing the environment for recycling of the complexes to the cytoplasm. Therefore, interactions of a variety of molecules result in the accumulation of cargo molecules inside the nucleus via the import pathway and their removal via their export to the cytoplasm.

4. NPC and Diseases

Structural changes in Nups and carriers or defects in transport pathways leading to nuclear or cytoplasmic overaccumulation of materials are correlated with a number of diseases, such as cancer, immune system disorders, and nervous system diseases. Sometimes, an alteration in the transport mechanism is the reason behind these types of diseases; while they could also caused by conformational changes or malfunctions appear as a result of an NPC disorder. A thorough understanding of the relation between NPC function and these diseases is a stepping stone toward the development of treatments for them.

4.1. Cancer

Overexpression of at least one of the NPC protein decoder genes likely induces cancer. For instance, overexpression of Nup88 (Fig. 6.2) located on the cytoplasmic side of the NPC is observed in breast and ovarian tumors (Cronshaw and Matunis, 2004). This Nup has cell-specific activities and its failure to control specific signal translocation pathways in human cells potentially gives rise to tumor formation. This Nup anchors Nup214 (Fig. 6.2) to the NPC and facilitates the export of NES-bearing cargos. Overexpression of Nup88 in cancerous cells decreases the NFκB export (i.e., the transcription factor involved in apoptosis, cancer, and immune responses) thereby, causes NFκB accumulation in the nucleus. This

increased concentration of NFκB in human cell nuclei, such as colon carcinoma, breast, and pancreas, induces a detrimental upregulation of target genes (Kohler and Hurt, 2010; Xu and Powers, 2009).

However, the NCT patterns are altered in tumor cells. The expression level of transporters, presumably affecting the transport patterns, also changes in cancer cells. Any mutation or expression level changes in transport receptors or RanGTP/RanGDP gradient results in changes the distribution of factors like tumor suppressors or oncoproteins (Chahine and Pierce, 2009). Overexpression of CAS is observed in many types of cancers, such as liver neoplasm, breast, and colon cancers. This overexpression increases nuclear concentration of impα, which introduces a redistribution of tumor suppressors and oncoproteins and thereby, an enhanced cell proliferation and resistance against apoptosis. An increase in concentration of CAS obstructs the import of P53 by impα (Chahine and Pierce, 2009; Kau et al., 2004). Yet it is not known whether this overexpression induces cancer via changes in the distribution of impα-dependent cargoes, like P53 (a tumor suppressor protein), or it is due to a secondary role of CAS independent of its participation in transport phenomena as an exportin.

A specific form of impα is observed in ZR-75-1 breast cancer cells, which lacks the NLS-binding domain. In such cells, because of impα structural defects, the nuclear import level of P53 is reduced and P53 remains in the cytoplasm. Accumulation of the cytoplasmic P53 was observed in 40% of breast cancer cases (Kim et al., 2000). In these cells, in addition to P53, other suppressors are likely to be affected by inappropriate localizations.

Upregulation of Ran occurs in prostate, breast, colon, kidney, ovarian, sarcoma, and nasopharyngeal carcinoma cancers. While a small magnitude of Ran suffices viability of normal cells, a significant increase in the amount of Ran concentration causes tumorigenesis. Abnormalities in Ran-gradient-regulating enzymes during oxidative stress have been observed in breast cancer. In other words, oxidants eventually destroy the Ran gradient pattern and nuclear import of proteins (Chahine and Pierce, 2009).

In "familial adenomatous polyposis" (a dominant autosomal disease characterized by development of colon carcinoma) due to defects in the adenomatosis polyposis coli gene, which induces CRM1-mediated-βcatenin export, β-catenine piles in the nucleus abnormally and consequently leads to the activation of transforming genes (Faustino et al., 2007; Henderson, 2000). In some cancerous cells, accumulation of cargoes in the nucleus or cytoplasm is caused by alterations in cargo NLSs or NESs, such as NFκB, whose activation induces tumorigenesis and cancers like Hodgkin's lymphoma. In healthy cells, NFκB joins a complex with IκB inhibitor, which masks NLS of NFκB to block its way to the nucleus. When IκB is phosphorylated and degraded, NLS of NFκB is unmasked and allows NLS to enter the nucleus. Hence, defects in NFκB regulators cause

upregulation of tumorigenesis target genes (Chahine and Pierce, 2009). Several cases of such inappropriate crowds of cancerous cells have been observed.

Genes of several Nups, which produce "oncogenic fusion proteins" (i.e., a protein made from a fusion gene when parts of two different genes join), are known to contribute to some types of cancers. For example, translocation of Nup98 gene (i.e., the movement of Nup98 gene fragment from one chromosomal location to another) is related to hematological malignancies, especially in acute myeloid leukemia (AML). Nup98 gene could fuse to at least 14 other genes. This Nup has an FG repeat sequence at the end of its N-terminus, which is the Rae1 binding site responsible for RNA export and the mitotic checkpoint activation. This part of Nup98 fuses to its partners, most importantly the homeobox family of transcription factors (HOX). A chromosomal rearrangement following that produces oncogenic fusion proteins, which induce tumorigenesis and cancer. These proteins potentially cause deficiency in the transport of materials, leading to leukemogenesis. It is also possible that Nup98 FG repeats randomly, via interaction with transcriptional coactivators, excite transcription of some leukemogenesis-related genes. Since Rae1 (a protein bound to Nup98) is a regulator of mitotic checkpoint activation, any deficiency of Rae1 could lead to a disturbance in the cell mitosis and thereby, leukemogenesis. Multiple mechanisms are speculated to engage in this transformation process. In some cancers, such as myeloid leukemia, fusion of Nup214 gene (an FG-Nup located on the cytoplasmic side of the NPC) (Fig. 6.2) has been reported. Nup214 gene could fuse to coder genes of DNA-associated proteins like DEK and SEK, generating new chromosomal translocations. Also, Nup214 FG repeat probably causes activation of other genes related to leukemia. Nup98 and Nup214 fusion proteins remain in the cytoplasm and preserve their capability of binding to soluble factors (Cronshaw and Matunis, 2004; Kohler and Hurt, 2010; Xu and Powers, 2009).

Sequestration of karyopherins obstructs their active participation in the transport cycle. Tpr is a 265-kDa Nup located on the nuclear side of the NPC and is a compound of the nuclear basket (Fig. 6.2). Tpr contributes to the export of RNA and proteins from the nucleus and regulates Mad1 and Mad2 checkpoints of the mitosis spindle. Biochemical studies show that the N-terminal coiled coil domain of this Nup fuses to kinase proteins such as MET, RAF, and TRK, and it mediates protein polymerization, activates protein kinases in cellular transformation, and plays a role in the occurrence of cancer (Kohler and Hurt, 2010; Xu and Powers, 2009). Another Nup that might be involved in chromosomal translocation is Nup358. This Nup could fuse its N-terminus to the kinase protein domain Alk and by activating Alk induce cell transformation (Ma et al., 2003).

As opposed to other fusion proteins, which are separate from the NPC, this oncogenic fusion protein is localized on the NPC.

4.2. Autoimmune diseases

In some autoimmune diseases (e.g., primary biliary, symmetric lupus arythroid, etc.), anti-NPC autoantibodies attach to some Nups, such as Nup62, Nup153, Nup358, gp210, and Tpr (Fig. 6.2). In primary biliary cirrhosis (PBC), bile ducts deteriorate gradually, resulting in the development of liver cirrhosis. Autoantibodies for gp210 and Nup-p62 (Fig. 6.2) recognized in patients suffering from these diseases, especially during the advanced stages, could be markers of how severely the disease has developed. However, looking at the strong correlation between the concentration of these antibodies and development of the disease, some researchers proposed that these antibodies themselves cause the disease, which still remains unverified (Cronshaw and Matunis, 2004; Tsangaridou et al., 2010).

4.3. Nervous system diseases

Central nervous system (CNS) selective effects of RanBP2 could prepare pathogenesis background for specific neuropathies, such as Parkinson. In this CNS disease, mutated Parkin protein has E3 ubiquitin ligase activity. It targets RanBP2 and the attached ubiquitin to RanBP2, and protosomal degeneration occurs in RanBP2 (Aslanukov et al., 2006; Um et al., 2006). Acute necrotizing encephalopathy (ANE) is another CNS disease that is diagnosed in young children usually after an influenza type A or B viral infection. It is suggested that this disease develops as a result of a mutation in RanBP2 (Gika et al., 2010; Huang et al., 2004; Neilson et al., 2009).

Recently, conducted studies on relations of NPC proteins and Alzheimer (a fatal brain disorder) reveal that a nuclear irregularity occurs in the NPC and Tau proteins (usually in association with neurofibrillary tangles). In addition, a cytoplasmic accumulation of NTF2 in hippocampal neurons (with or without tangles) is observed in Alzheimer, indicating the inharmony of the transport. Also, disruptions in the distribution pattern of some karyopherins like impα1 are reported in Alzheimer (Lee et al., 2006; Sheffield et al., 2006; Yadirgi and Marino, 2009).

Triple A syndrome (also called as Allgrave syndrome) is an autosomal recessive disorder characterized by adrenal failure, achalasia of the cardia, alacrima (absence of tears), and neurological defects. Triple A syndrome is a consequence of a mutation in ALADIN (alacrima achalasia adrenal insufficiency neurological disorder) gene, a part (Nup) of the NPC

structure. However, recent investigations indicate that ALADIN integration in the NPC occurs via the NDC1 transmembrane Nup. Since reduction of the ALADIN integration is a major mechanism for triggering and the development of Triple A syndrome, it is suggested that the interaction between these two Nups might play a role in pathogenesis and its elimination might be important in development of the disease. An initial defect in ALADIN protein disrupts the karyopherin α/β-mediated import pathway and hence, blocks the nuclear import of DNA report proteins like aparataxin and DNA ligase I. This makes DNA damage and cellular death under the oxidative stress highly probable. Further, as a result of ALADIN Nup defects, nuclear import of Ferritin heavy chain, which is the DNA protector, is disrupted, making the cell more prone to the oxidative damage. Engagement of adrenal and CNS is clearly observed in this disease (Kind et al., 2009; Kiriyama et al., 2008; Storr et al., 2009; Yamazumi et al., 2009).

Mutation in Nup62 gene, which is the coder gene of one of Nups called p62, gives rise to a disease named infantile bilateral striatal necrosis (IBSN) that is a neurodegenerative disorder, and CNS is engaged in this disorder in the same way as it is in Triple A syndrome. p62 taking part in the NCT plays a cell type-specific role in basal ganglia degradation (a group of nuclei in vertebrate brains). Reportedly, mutations of protein gene do not affect its localization in the NPC and they are cell-type-specific mechanisms caused by these mutations that induce the disease. However, this is a matter of controversy and various hypotheses, such as partial abnormality of the NPC structure, detrimental disorders in transport pathways of proteins involved in neural cells in basal ganglia, and alteration in chromatin localization in some specific cells, exist on trigger of this disease (Basel-Vanagaite et al., 2006; Chahine and Pierce, 2009).

Amyotrophic lateral sclerosis (ALS) is an incurable progressive disease affecting a group of motor neurons in the brain and spinal cord, resulting in neurodegradation as well as muscle degradation. Studies on the cells suffering from this disease, i.e. anterior horn cells (AHC), show irregularities of nuclear contours along with an inefficient distribution of transporters along the channel. Lack of impβ and irregularity in Nup62 in a subset of these cells suggested that a detrimental dysfunctional NCT occurs in these cells. Again, like Triple A syndrome, disruptions in transport activities of the NPC most probably hinder a successful entry of a regeneration signal to the nucleus, facilitating the neurodegradation and taking part in the ASL pathogenesis in this way. Transactivation response DNA binding protein 43 (TDP43), which typically exists in the nucleus, has been observed only in the cytoplasm of diseased cells. It is assumed that their disease is caused by the NPC dysfunction and is related to the ASL pathogenesis (Kinoshita et al., 2009).

4.4. Cardiac disease

A direct link between the NPC machine and heart disease was recognized first in arterial fibrillation (AF), which is a cardiac dysrhythmia. Further studies revealed that mutations in Nup155 coder gene (a Nup involved in transport of mRNA and proteins) lead to the development of this disease and early sudden cardiac death. Decrease of Nup155 interrupts export of HSP70 mRNA and import of HSP70, which is a vital protein for the cell, to the nucleus. Therefore, it is suggested that Nup155 plays a key role in regulating HSP70 gene expression and mutation in this gene causes cardiac death. In addition, recent studies focusing on the influence of heart failure (HF) on the NPC in human cardiomyocytes indicated high levels of importins, exportins, Ran regulators like RanGAP, and p62, whereby density of NPCs did not show any significant change (Cortes et al., 2010; Zhang et al., 2008).

4.5. Infectious diseases

Another category of diseases that could be stemmed from the NPC and its function is infectious diseases. Generally, viruses fall into two categories of those including DNA genome and those including RNA genome, each of which deals with the NPC in a distinct way to cause the disease.

4.5.1. Viruses with DNA genome

Viruses encapsulating DNA genome, after entering the cytoplasm, should pass through the NPC to access the nucleus to fuse their genes to the host cell genes and proliferate. The major obstacle on their way to the nucleus is the small diameter of the NPC. Small viruses, such as hepatitis-B virus (HBV) and parvovirus (MVM), easily travel through the NPC with their whole viral capsid, but larger ones, like herpes simplex virus (HSV), need to disassemble their viral capsid to be able to pass across the NPC (Chowdhury, 2009; Greber and Fornerod, 2005; Puntener and Greber, 2009).

Transport of HBVs through the NPC is facilitated by phosphorylation of their capsids during maturity, which are actually their NLSs. This change in the viral capsid eases access of NLS to impα and impβ. In the nuclear basket, upon interaction between impβ and Nup153, the entering complex is stopped whereby RanGTP unbinds the transporter (impβ) and recycles it back to the cytoplasm. When the transporter is unbound, viral capsids attach to the Nups 150 times stronger than impβ does. Thereafter, mature capsids become disassembled and capsid subunits and viral DNAs leave the nuclear basket and get released in the nucleus, while immature capsids are trapped in the nuclear basket, waiting for further maturation (Schmitz et al., 2010). Large viruses like HSV or adenovirus particles primarily attach to cytoplasmic fibers. Docking of HSV through the NPC relies on the presence of

impβ, and once inside the nucleus, HSV releases its DNA (Lee and Chen, 2010; Rixon, 2010). However, adenovirus capsids in the cytoplasm bind to CAN/Nup214 directly. These viruses trap necessary factors for their capsid disassembly, such as Hsc70, histone H1, and transport factors of impβ and importin7 (Greber and Fassati, 2003), and finally release their DNA into the NPC to travel all the way to the nucleus (Russell, 2009; Trotman et al., 2001). Recent studies suggested that protein VII and its receptor, transportin, mediate import of DNA (Hindley et al., 2007). Also, polyomaviruses like SV40 undergo changes before reaching the nucleus. These viruses release their DNA containing subviral particles in the cytosol by passing through the endoplasmic reticulum, which causes exposure of some capsid protein NLSs, and these NLSs enter the nucleus by mediation of impα and impβ (Puntener and Greber, 2009). Papillomaviruses are another category of viruses that become uncoated in the endoplasmic network prior to entering the nucleus and they need importins to be transported to the nucleus because of their large size (Sapp and Day, 2009).

4.5.2. Viruses with RNA genome

Viruses with RNA, for example, picornaviruses (polioviruses, rhinoviruses) and rhabdoviruses, proliferate in the cytoplasm of their host cells and they do not need to reach the nucleus. Nevertheless, cells infected by these viruses experience NPC structural and functional nucleocytoplasmic disorders. For instance, infection by polioviruses or rhinoviruses causes certain nuclear proteins, such as Sam68, La, and nucleolin, to become jammed in the cytoplasm (Hiscox, 2003; Weidman et al., 2003). These proteins induce virus replication upon reacting with its RNA. In fact, these viruses inhibit active import by the proteolytic degradation of Nups, especially Nup62, Nup153, and Nup98, and thereby weaken the host cell's immune response against the virus (Gustin and Sarnow, 2002; Park et al., 2008). Most probably, the overall activity of the NPC continues after infection because certain imports and exports keep occurring even while the cell is infected (Lin et al., 2009).

Vesicular stomatitis virus (VSV) includes an important protein called M-protein. This protein induces some alterations in the NPC and the transport pathways. It is thought to interact with Nup98 on the nuclear side of the NPC and is capable of dissociating Rae1 from Rae1–Nup98 complex and inhibiting the mRNA export process. It also obstructs nuclear import processes and the export of UsnRNA, rRNA, and snRNA. During the antiviral response via interferon signaling, Nup98 becomes upregulated thereby, releases M-protein and commences the export process (Cronshaw and Matunis, 2004; Ren et al., 2010; von Kobbe et al., 2000).

Although retroviruses are mostly able to proliferate in dividing cells, which lack a nuclear envelope, a group of them called lentiviruses (including HIV) proliferate in nondividing cells as well, because these viruses gain

access to their host cell DNA upon passing across the NPC. These viruses lose their capsid in the host cell cytoplasm and consequently, their RNA genome is reverse-transcribed into a complementary DNA (cDNA). This cDNA along with cellular and viral protein factors forms a complex termed preintegration complex (PIC), which enters the nucleus through the NPC and obtains proliferation capability. This complex contains matrix protein (MA), Vpr, integrase (IN), and DNA flap which are able to mediate the DNA nuclear import either directly or by means of karyopherins, such as impα, impβ, imp7, and transportin, or by means of different Nups, such asNup98, Nup358, Nup153, and RanBP2. It has been shown recently that a direct interaction between integrase and Nup153 takes part in the PIC nuclear import (Woodward et al., 2009). It appears that MA, Vpr, and IN proteins enter the nucleus via interaction with importins and Nups (Aida and Matsuda, 2009; Suzuki and Craigie, 2007; Suzuki et al., 2009). Most likely, central DNA flap plays a more important role in the import process than do other elements (De Rijck and Debyser, 2006; Riviere et al., 2010). Interestingly, it is suggested that HIV docks to cytoplasmic fibers on the NPC by its capsid upon arriving in the cytoplasm and the reverse-transcription on its RNA confers a cDNA, and finally, upon formation of a DNA flap, PIC enters the nucleus after removal of the viral capsid. In other words, in this model, the viral capsid has the central role in the cDNA nuclear import (Arhel et al., 2007; Suzuki and Craigie, 2007; Zennou et al., 2000).

Avian sarcoma virus (ASV), which is an alpharetrovirus, is able to penetrate cycle-arrested cell nuclei. Apparently, this virus enters its host cell nucleus by a protein on its integrase. Studies indicate the ASV integrase import occurs without a classic binding to impα but depends on soluble cellular factors. Integrase enters the nucleus by exploiting one or a few soluble cellular factors responsible for transport of histone H1 (Anderson and Hope, 2005; Andrake et al., 2008; Chowdhury, 2009).

Influenza virus is an Orthomyxovirus containing eight genomic ribonucleoproteins (RNPs), including RNA and nucleoprotein (NP). This virus is equipped with RNA-dependent RNA polymerase, which is an enzyme for replication and transcription. In a certain level of infection, RNPs and some other viral proteins are released in the cytoplasm, whereas they need to enter the nucleus to be able to proliferate. Generally, during an influenza infection, several nuclear imports and exports occur. In primary levels of infection, incoming VRNPs wandering in the cytoplasm enter the nucleus, and afterward, synthesized transcripts of the virus are exported from the nucleus. Then, newly synthesized proteins, NPs and RdRp subunits (PA, PB1, and PB2), enter the nucleus via their specific NLSs, and finally, Crm1 helps assembled VRNPs exit the nucleus. RNA to be transported to the nucleus is dependent upon the presence of impα, impβ, and Ran, as well as a coating with nontypical NLS-carrying NPs. It is yet to be known that whether these specific NLSs bind directly to RNA or they attach

to the transporters. Recently, a lot of attention has been paid to investigating the nuclear transport mechanism of influenza virus proteins. For instance, it was determined recently that in influenza A virus (a specific type of influenza virus), PB1 and PA forming a dimer, enter the nucleus via importin5 factor. PB2 separately penetrates the nucleus and attaches to PA–PB1 dimer in the nucleus. A mutation in the PB2 NLS subunit hinders its nuclear import, leading to the formation of an abnormal polymerase in the cytoplasm. Additionally, in influenza A virus, two NLSs (NLS1 and NLS2) on the NP facilitate the transport of RNPs into the nucleus. Studies show that for incoming VRNPs and newly distributed NPs, to enter the nucleus, their NLS is exposed to the interaction. After the NPs entered the nucleus, RNP assembly occurs and RNPs are exported from the nucleus and these RNPs are no longer able to enter the nucleus. Among these RNPs, it is observed that NLS1 is hidden and thus, it is suggested that NLS1 hides in the NPs prior to the RNP assembly. Nonetheless, another model states that NLS1 hides after the NPs are assembled to form the RNP. Experiments indicated that the NP oligomerization is not the reason behind this NLS masking, rather it happens because of a past modification, binding to cellular proteins or the NP conformational change. Therefore, NLSs play a significant role in the function of these viruses and they regulate the nuclear transport directionality of their genome via selective exposure of NLSs (Whittaker et al., 1996; Wu and Pante, 2009).

Basically, each cell shows a specific reaction when it faces a virus attack. For this response to develop, transcription factors should enter the nucleus and activate some particular genes. A group of alphaviruses take a smart action to prevent the host cell from showing this response, that is, blocking the NCT pathway. Venezuelan equine encephalitis virus (VEEV) blocks the NPC central channel to stop the import of gene transcription factors needed to induce the response against the virus. The capsid of this virus binds simultaneously to Crm1 exportin, impα, and impβ in the cytoplasm, forming an abnormal tetrameric complex. Also, these capsids are able to make dimeric complexes with Crm1 and attach to Nup358 and finally, they form a tetrameric complex with impα and impβ. This odd complex settles at the NPC central pore and blocks import of crucial factors to the nucleus, though small proteins can still shuttle into and out of the nucleus. Using this strategy, the virus stops the cell antiviral response and increases its ability to proliferate (Atasheva et al., 2010).

4.6. Other disorders

Cell stressors such as UV irritation, oxidative stress, and heat shock stress induce diseases like schema, HF, hypertension, diabetes, and cancer by mislocalizing transport receptors like impα (Chahine and Pierce, 2009; Miyamoto et al., 2004). Additionally, Ran overexpression interrupting

nuclear retention of important transcription factors engaged in activation of T-cells potentially causes defected responses in these cells (Qiao et al., 2010).

In addition to the aforementioned disorders, an import pathway has been discovered that participates in determining the sex by gender determination transporting transcription factors. This conserved pathway among eukaryotes is mediated by Ca^{2+}/calmodulin and is independent of Ran. It is known that defects in this NPC pathway due to stopping transit of some factors such as SRY and SOX9 in sertoli cells could cause human sex reversal diseases like campomelic dysplasia and sywer syndrome (Hanover et al., 2009).

5. Conclusion

The purpose of this review was to examine the nucleocytoplasmic pathways, interaction of molecules taking part in transport processes, and NPC-related diseases. In the past few years, major scientific efforts have been made to express and analyze the sophisticated structure of the NPC, the bilateral NPC pathways, and the roles played by molecules involved in the NCT process, which are critical to understanding how this super-efficient nanomachine works and how it may potentially control the mechanobiology of the cell (Wolf and Mofrad, 2009). These studies, conferring an overall insight of the NPC function, have set the stage for us to move on to more detailed investigations in this field. Several diseases such as cancer, neural and immune system, and infectious diseases have been associated with NPC structural disorders and NCT disruptions. Further study of this dependence can shed light into the mechanism of this complicated system, and once this mechanism is deciphered, we will be able to predict and control transport of macromolecules more accurately to come up with affordable treatments for such diseases. In other words, on one hand, knowledge of the NPC structure and function paves the way toward understanding diseases and thereby discovering efficient treatment methods for them, while on the other hand, study of diseases increases our understanding of the NPC.

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