Nucleocytoplasmic Transport: The Last 200 Nanometers

Review

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Introduction

More than one million macromolecules per minute are actively transported between the nucleus and cytoplasm of a growing mammalian cell. This bidirectional traffic is routed through nuclear pore complexes (NPCs), structures that penetrate and fuse the double bilayer membrane of the nuclear envelope (NE). They form aqueous channels through the NE in a manner superficially similar to the way a buttonhole can be used to join two layers of cloth and simultaneously form a passage. Unlike a buttonhole, however, the NPC is an active participant in nucleocytoplasmic transport. Whereas globular proteins of greater than 60 kDa cannot cross the NPC by diffusion at a significant rate, the largest known substrates for active transport are roughly 25–50 megadaltons (MDa). The diameter of the NPC channel through which active transport occurs opens to a maximum of approximately 25 nm, but the diffusion channel is 9 nm, indicating that the NPC recognizes and reacts to specific transport substrates by undergoing a considerable change in conformation.

As expected from the size of substrates that can pass through them, NPCs are large. Vertebrate NPCs are close to 30 times the mass of a ribosome, 125 MDa (Reichelt et al., 1990), and yeast NPCs are at least 66 MDa (Rout and Blobel, 1993). This size and the accompanying complexity of NPC composition are considerable barriers in understanding NPC function. Our review summarizes information on NPC structure and composition and discusses progress in understanding the function of its components, the nucleoporins, in translocating substrates across the 100–200 nm from one extreme of the NPC to the other.

Substrates, Adapters, and Receptors: A Summary

To put the NPC in context, we start with a description of the complexes that are translocated through it. Substrates for nuclear import or export carry a transport signal. In the simplest case, the signal is bound by a transport receptor on one side of the NPC, translocated through the NPC via receptor-mediated interactions, and released on the other side. The empty receptor is then recycled to the original compartment and restored to a form competent for signal binding (Figure 1). A common and slightly more complicated variant of this scheme involves the use of adapters. In these cases, substrate–receptor interactions are not direct but are mediated by one or more adapter proteins. Here, not only the receptor but also the adapter has to be recycled after transport of the substrate and therefore also shuttles between the nuclear and cytoplasmic compartments.

The data that gave rise to this skeletal description of transport events has been reviewed extensively in the recent past (Görlich, 1997; Nigg, 1997; Ullmann et al., 1997; Mattaj and Englmeier, 1998). We refer the reader to these reviews for more detailed explanations and original references. For our purposes, it will suffice to briefly describe some facets in slightly more detail.

A characteristic of nucleocytoplasmic transport is directionality. Substrates are moved either into the nucleus or out of it. Ran, a small GTPase, is thought to be critical for this directionality. Like other GTPases, Ran needs regulators for its activity. The regulators either stimulate Ran to hydrolyze GTP or to release the resultant GDP and rebind GTP. Ran's regulators are distributed asymmetrically across the NE such that GTPase activity is favored in the cytoplasm and GDP/GTP exchange in the nucleus. This predicts a high nuclear RanGTP concentration and a low cytoplasmic one. This inequality was proposed to help establish the identity of the two compartments (Görlich et al., 1996a; 1996b) as described in the following examples.

The first involves CRM1 (or exportin 1), a receptor that functions in the export of a group of proteins that carries a specific, leucine-rich type of nuclear export signal (NES). CRM1 binds to its substrate and to RanGTP cooperatively, and NES interaction is only significant in the presence of RanGTP (i.e., in conditions found in the nucleus). After export, the GTP in the RanGTP-CRM1-NES protein complex is hydrolyzed, causing the NES to dissociate from CRM1. CRM1 can then be reimported to the nucleus in the empty state (Figure 1A). In this example, CRM1 functions without an adapter. In some cases NES-containing proteins bind to RNAs that are destined for nuclear export. In these cases, the NES proteins are adapters, and the transported substrate is the RNA.

Importin β, our second example, is the import receptor for proteins that carry a classical nuclear localization signal (NLS). As shown in Figure 1B, Importin α is an adapter that mediates formation of an import complex, including the NLS-containing protein and Importin β. This complex translocates through the NPC into the nucleus, where it meets RanGTP. RanGTP binding to Importin β causes Importin α and the NLS protein to dissociate (Figure 1B). Empty Importin β, probably still bound to RanGTP, is then reexported. Recycling of the adapter, Importin α, requires a specific export receptor, CAS, that is analogous to CRM1. CAS interaction with Importin α requires cooperative RanGTP binding, and CAS prefers Importin α, which is not associated with an NLS. Thus, empty Importin α is reexported by CAS. After hydrolysis of the Ran-bound GTP in the cytoplasm, Importin α dissociates, and CAS can be returned to the nucleus (Figure 1B), resetting the mechanism for import of an additional NLS-containing protein.

The three receptors in our examples, CAS, CRM1, and

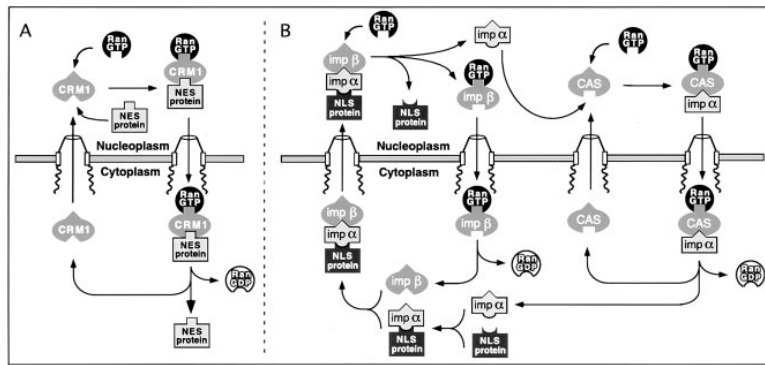†To whom correspondence should be addressed.

Figure 1. Two Examples of Receptor-Mediated Transport of Soluble Factors between the Cytoplasm and the Nucleus

(A) In the nucleus, an NES-containing protein binds to CRM1 cooperatively with RanGTP and is subsequently translocated through the NPC. In the cytoplasm the complex dissocates when Ran hydroloyzes GTP under the influence of RanBP1 or 2 and RanGAP1. Empty CRM1 then reenters the nucleus.
(B) In the cytoplasm, an NLS protein binds to Importin β via the adapter Importin α and is translocated to the nucleus. There the complex dissociates on RanGTP binding to Importin β. CAS is the export receptor for Importin α and is analogous to CRM1 in (A). Both CAS and Importin β-RanGTP in the empty state must be recycled.

Importin β, all need to interact with both RanGTP and with NPC components involved in translocation. It is thus not surprising that they are members of a family of related proteins (Fornerod et al., 1997b; Görlich et al., 1997) (Figure 2). Thus far, six members of the family are known to be export or import receptors, and it is likely that the others will have this function. Note that it is not unlikely that a given receptor could function in the import of some substrates and in the export of others. There are currently no reports of nucleocytoplasmic transport receptors from outside this family.

The interactions between soluble transport factors described in these examples are still not fully understood. However, this brief introduction permits a better definition of what is important for the transport steps that involve the NPC. In the cases studied so far, it is the receptors that are the targets of NPC recognition and NPC translocation, whereas adapters and substrates seem to behave as inert cargo. We will return to NPC translocation after describing the structural organization of NPCs.

Overall NPC Architecture

NPC structure has been analyzed by a variety of electron microscopic techniques (Figure 3) (Akey and Radermacher, 1993; Panté and Aebi, 1994; Rout and Wente, 1994; and Goldberg and Allen, 1996, for reviews and different NPC models). The favored source of NPCs has been amphibian oocytes, but many aspects of the deduced structure are conserved in insect and yeast NPCs. The membrane-spanning part of the NPC consists mainly of an 8-fold symmetrical arrangement of spoke structures. These form a ring in which sits the central plug, or transporter, through which active transport occurs (Feldherr et al., 1984; Akey and Goldfarb, 1989). The spokes penetrate into the lumen of the nuclear envelope and are therefore presumed to have transmembrane components and to be essential for anchoring the NPC in the membrane.

On both the cytoplasmic and nuclear surfaces of the ring of spokes are annular structures that are of similar but not identical size and shape. These are called the
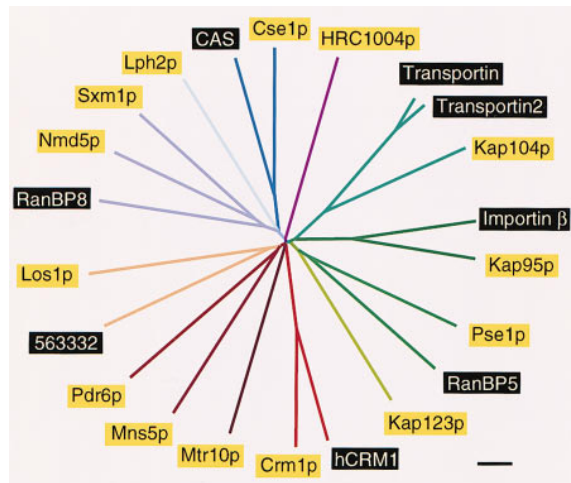

Figure 2. The Importin β Family of Transport Receptors

Yellow, *S. cerevisiae* proteins; black, human proteins. Kap95p is yeast Importin β; Kap104p is yeast transportin. The ClustalX alignment was generated using the most conserved region, the 300 N-terminal amino acids, because some branches were unstable when the complete sequences were used. 563332 is a cDNA from the EST database with accession numbers AA113097 and AA112285. (For other accession numbers, see Fornerod et al., 1997b; Görlich et al., 1997). Bar, 10% sequence divergence.
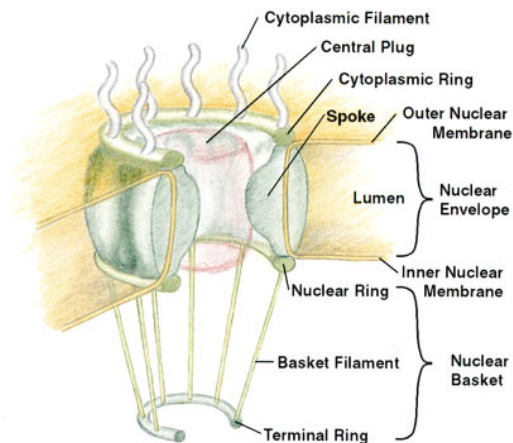

Figure 3. A Cut-Away Model of an NPC

cytoplasmic and nuclear rings. The cytoplasmic ring carries eight cytoplasmic filaments that extend at least 30–50 nm into the cytosol. The nuclear ring supports eight thinner fibers of roughly 100 nm that are joined by a terminal ring, forming a structure called the nuclear basket (Figure 3). Not all of the NPC structures described are uncontroversial (Panté and Aebi, 1994), and it remains to be seen which aspects of NPC structure are functionally important.

The ensemble of these structures is considered to be the NPC, but elements of vertebrate NPCs are attached to other nuclear structures. The nuclear ring is attached directly to the nuclear lamina, a network of intermediate filament-like structures comprised by the lamin proteins that extend over the inner surface of the nuclear envelope (Aaronson and Blobel, 1975; Aebi et al., 1986). The terminal ring of the nuclear basket contacts long filaments that contain the p270/Tpr protein. The filaments are arranged as though on the surface of a cylinder and extend deep into the nucleoplasm (Cordes et al., 1997, and references therein). In addition, a distinct filamentous structure called the nuclear lattice attaches to the terminal ring (Goldberg and Allen, 1992). This gives the impression that NPCs might be part of a rigid nuclear structure. As we will see later, this impression may be incorrect.

The most divergent source of comparative information on NPC structure are biochemically enriched yeast (Saccharomyces cerevisiae) NPC preparations (Rout and Blobel, 1993). These retain the 8-fold symmetrical spoke structure surrounding a central plug, suggesting that basic aspects of the membrane-spanning part of the NPC are conserved. Recent examination of less purified yeast NPCs has provided evidence that the cytoplasmic filament and nuclear basket structures are also present (Fahrenkrog et al., 1998).

NPC Composition

Estimates of the number of distinct NPC components vary from 50–100, and a recent review listed nearly 30 characterized yeast nucleoporins (Doye and Hurt, 1997). It is therefore to be expected that yeast nucleoporins will soon all be identified. Progress with vertebrate nucleoporins is steady but slower. It will be helped by the genome projects and the yeast data, although the level of conservation of nucleoporins is remarkably low. Several characterized vertebrate nucleoporins have no homolog in the yeast genome, and even those that do generally exhibit a very low level of sequence identity.

There are some common features of nucleoporins. A subset contains repeated motifs that end in the dipeptide FG. These repeats come in at least three distinct varieties, according to their sequence composition (Rout and Wente, 1994; Doye and Hurt, 1997). The presence of such repeats in a novel protein can be considered predictive but not diagnostic that the protein is a nucleoporin. There are indications (see below) that these repeats may be sites of interaction with transport receptors. In addition, vertebrate nucleoporins often carry O-linked N-acetyl glucosamine modifications (Holt et al., 1987), although neither the extent of conservation of this modification in other species nor its function is known.

NPC Subcomplexes

The presence of distinct structural elements within the NPC suggests the existence of NPC subcomplexes, and there is in fact direct evidence for these. A simple example contains two components of vertebrate NPC cytoplasmic filaments, CAN/Nup214 and Nup88, that coimmunoprecipitate from cell extracts (Bastos et al., 1997; Fornerod et al., 1997b). Another component of the cytoplasmic filaments, RanBP2/Nup358 (Wu et al., 1995; Yokoyama et al, 1995), does not copurify, indicating that the subcomplex is not identical with the filament structures. A well-studied example is the p62 complex. In Xenopus laevis, this consists of three glycoproteins, p62, p58, and p54, whereas the rat complex also contains p45 (Finlay et al., 1991; Guan et al., 1995). The p62 complex is located on both sides of the NPC at or near the ends of the central plug and may therefore play a role in recognition or translocation of transport substrates (Hu et al. 1996, and references therein). Consistent with such a function, NPCs assembled without the p62 complex do not have obvious structural defects but are incapable of nuclear protein import (Finlay and Forbes 1990, Finlay et al., 1991).

An analogous complex exists in yeast. It includes Nsp1p (Nehrbass et al., 1990), which has a similar domain organization to p62 and may be its homolog; two additional FG repeat nucleoporins, Nup49p and Nup57p (Grandi et al., 1995; Hu et al., 1996); and Nic96p (Grandi et al., 1995). In biochemically isolated yeast NPCs, Nic96p is one of five particularly abundant proteins, the others being Nup170p, Nup157p, Nup188p, and the transmembrane nucleoporin Pom152p (Aitchison et al., 1995). Nup188p interacts with both Pom152p and Nic96p and is detectable on both faces of the pore (Nehrbass et al., 1996; Zabel et al., 1996). There may therefore be a network of interactions from the membrane-associated Pom152p over Nup188p and Nic96p to the Nsp1p complex. Extending the cross-species analogy, Xenopus p62 interacts directly with Nup93, the Xenopus Nic96p homolog (Grandi et al., 1997). Extension of this type of genetic and biochemical bootstrapping between nucleoporins and between species should help to create a picture of the composition of conserved NPC substructures. NPC subcomplexes may either be units of specific function or substructures that are preformed during NPC assembly.

NPC Assembly and Dynamics

There are two situations during which NPC insertion into the NE occurs. In all cell types, new NPCs must be incorporated into the NE during growth. Additionally, in cells with an open mitosis, NPCs are disassembled and have to be reinserted into the reforming NE. It is not known whether the two types of NPC insertion are mechanistically identical. An extreme example of new NPC insertion are amphibian oocytes. Immature oocytes (oogonia), like somatic cells, have a few thousand NPCs, whereas mature oocyte nuclei have roughly 10^7. These NPCs are dismantled when the nuclear envelope breaks down on oocyte maturation, creating a large store of NPC building blocks in the egg. This store has been exploited in studies of nuclear, nuclear envelope, and
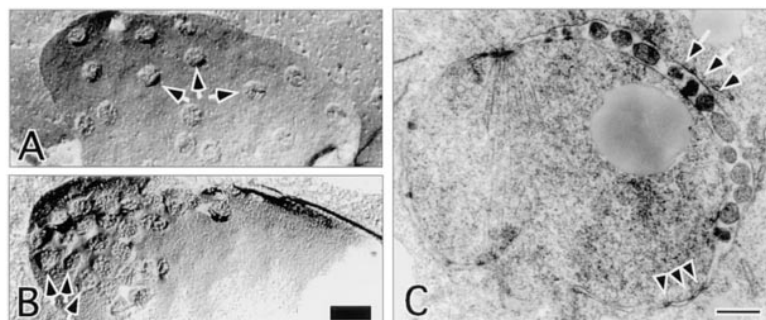
Figure 4. Yeast Nucleoporin Mutant Phenotypes Studied by Electron Microscopy

(A) Normal distribution of NPCs (arrows) in a *nup49* mutant cell; (B) NPC clustering in a cell lacking Nup133p. Note that these cells are temperature sensitive, but clustering is seen even at temperatures permitting growth, as shown here.

(C) NPC herniations (arrows) in a cell lacking Nup116p grown at the restrictive temperature (Bailer et al., 1998). Seemingly normal NPCs are indicated by arrowheads. Bar, 0.5 μm.

NPC assembly in vitro (reviewed by Wilson and Wiese, 1996; Macaulay and Forbes, 1996a). Inhibitor studies have been used to demonstrate that NE and NPC assembly can be divided into biochemically distinct steps (Macaulay and Forbes, 1996b). Scanning electron microscopy has begun to define structural intermediates in NPC assembly (Goldberg et al., 1997), including those that accumulate in the presence of inhibitors. In addition to providing insight into NPC assembly, these studies represent a promising way to obtain independent confirmation of the structural makeup of NPCs.

New NPCs may well be assembled from the nuclear membrane outward (Wozniak et al., 1989). In other words, an early stage in NPC assembly might involve transmembrane nucleoporins. A small number of such proteins, gp210 and POM121 from vertebrates (Wozniak et al., 1989; Greber et al., 1990; Hallberg et al., 1993), and Pom152p from yeast (Wozniak et al., 1994) have been identified. A short fragment of gp210 including the transmembrane domain was sufficient for accumulation at NPCs (Wozniak and Blobel, 1992). This indicates that gp210 might assemble with other transmembrane nucleoporins. Note, however, that how nucleoporins accumulate at sites of existent NPCs may not be closely related to the mechanism of insertion of new NPCs into the NE. gp210 has a second nucleoporin interaction domain that projects out of the membrane into the "cytosolic" region of the NPC, providing a possible site of soluble nucleporin attachment once gp210 has been localized within the membrane (Wozniak and Blobel, 1992).

The possibility to look at yeast nucleoporin mutants is of great potential for studies of NPC assembly. Although the highest resolution microscopy techniques cannot yet be applied to yeast NPCs, combinations of genetic manipulations with other microscopic methods have provided several surprising insights into NPC assembly and dynamics. The largest initial surprise was the observation that mutation of nucleoporins can cause gross alterations in NPC and NE morphology (Figure 4). Broadly, these effects can be divided into two classes. First, NPCs, which normally distribute randomly over the NE surface, can become clustered at one location (Doye et al., 1994; Li et al., 1995) (Figure 4B). This phenotype has been observed upon mutation of several yeast nucleoporins (Doye and Hurt, 1997). Mutation of a *Drosophila melanogaster* lamin protein, Dm$_o$, also leads to NPC clustering (Lenz-Böhme et al., 1997). Second, deformations in the pore-associated membrane region can

occur and cause outgrowth of NE membranes into or out of the nucleus. This can even result in the growth of a complete membrane seal over NPCs, rendering them useless (Wente and Blobel, 1993; Siniossoglou et al., 1996) (Figure 4C).

Although the basis of these phenotypes is not understood, each class of effect has particular implications. An important question related to NPC clustering was whether it reflected an assembly defect or was a consequence of movement of NPCs already in the membrane. Making use of green fluorescent protein-nucleoporin fusions, two different groups recently reported on NPC dynamics in living yeast cells (Belgareh and Doye, 1997; Bucci and Wente, 1997). They studied the movement of NPCs following nuclear fusion in mated yeast cells and the redistribution of NPCs into clusters in nucleoporin mutant cells. Both studies concluded that yeast NPCs are not fixed in position in the NE but rather move around rapidly in the plane of the membranes. These dynamics were unexpected because NPC-associated structures like the nuclear lamina, nuclear lattice, and the long, Tpr-containing filaments might have been expected to anchor NPCs in place. Because these NPC-associated structures may not exist in yeast, investigation of the mobility of vertebrate NPCs should be a high priority.

The second class of phenotype, pore-associated membrane deformations (Figure 4C), may also be revealing of nucleoporin function. NPC insertion involves fusion of the two bilayers of the NE to create a continuous seal around an aqueous hole. It is possible that faults in controlling this process lead to the membrane defects. In this respect, a further yeast NPC subcomplex that contains six nucleoporins, including Sec13p and the related Seh1p, is of particular interest (Siniossoglou et al., 1996; Doye and Hurt, 1997).

The creation of the pore membrane domain (Figure 3) from the two NE membranes has topological similarity to the creation of a membrane-bound vesicle, as occurs during vesicle transport from the endoplasmic reticulum or Golgi apparatus. Sec13p is part of the machinery required for formation of membrane-bound vesicles in the endoplasmic reticulum. The presence of Sec13p and Seh1p in an NPC subcomplex is therefore provocative. Indeed, mutation of several of the components of the Sec13p nucleoporin complex leads to either nuclear envelope deformities or NPC clustering (Doye and Hurt, 1997), consistent with the idea that this complex might interact with the pore membrane.

A final fascinating phenotype related to membrane

insertion is caused by mutation of Act2p, a divergent form of actin. This protein is found in both the nucleus and cytoplasm but not at the NPC (Yan et al., 1997). Mutation of Act2p leads to the accumulation of NPC material at the NE in the apparent absence of aqueous channels. These defects appear rapidly after Act2p inactivation, perhaps indicating that the NPCs are reextruded from the NE, allowing it to reseal (Yan et al., 1997). Information on how Act2p mutation generates this phenotype is awaited with interest.

To summarize, there is abundant evidence for subcomplexes of the NPC and some information on their composition, but it is not known whether they form prior to arrival at the site of NPC assembly. Multiple yeast nucleoporin mutants have unexpected effects on either NE morphology or NPC distribution, but how these mutant phenotypes relate to the normal functions of the nucleoporins is unclear.

How Many Steps in NPC Translocation?

NPC translocation must involve interaction between nucleoporins and transport receptors, the members of the Importin β family introduced earlier (Figures 1 and 2). For Importin-mediated import of proteins with a basic NLS, a transport complex that contains at least Importin α and β and the NLS-containing protein assembles. The first interaction of the transport complex with the NPC, or docking, seems to occur on the cytoplasmic filaments (Figure 3). From there, the complex is transferred to the center of the cytoplasmic face of the central plug (Feldherr et al., 1984; Akey and Goldfarb, 1989; Panté and Aebi, 1996). Transfer may involve several sequential interactions along the filament or initial binding to one of several sites on the filaments (Feldherr et al., 1984; Newmeyer and Forbes, 1988; Richardson et al., 1988). Alternatively, a single productive step involving filament bending to present the transport complex bound at the tip of a cytoplasmic filament to the central plug may occur (Panté and Aebi, 1996). Docking is energy-independent. However, there is not a general agreement as to whether transfer to the central plug requires energy.

Movement through the central plug is energy dependent but otherwise mysterious. Subsequently, the Importin transport complex interacts with sites within the nucleus, probably on the nuclear basket (Feldherr et al., 1984; Panté and Aebi, 1996). A mutant form of Importin β that cannot interact with RanGTP appears to bind irreversibly to some of these intranuclear sites (Görlich et al., 1996b). This led to the suggestion that they may represent the location of the final step in Importin-mediated transport, where RanGTP binding to Importin β would release Importin α and the NLS protein into the nucleoplasm and simultaneously cause Importin β to dissociate from the NPC and begin its outward translocation (Görlich et al., 1996b; Kutay et al., 1997a). This Importin β mutant is a dominant negative inhibitor of many forms of nucleocytoplasmic transport (Kutay et al., 1997a), suggesting that the sites to which it binds may be required by multiple import and export receptors. Alternatively, some mechanism might prevent any translocation occurring when these sites are occupied. Such a control mechanism may be required to coordinate import and export events through the same NPC.

Among the best characterized export substrates are the giant Balbiani ring (BR) messenger RNPs of *Chironomus* (Daneholt, 1997). The RNPs fold into a large compact structure during and immediately after BR RNA transcription and cross the nucleoplasm in this form. Once at the NPC, the BR RNPs dock at the terminal ring of the nuclear basket (Kiseleva et al., 1996). They are too large in their folded state (50 nm, diameter) to proceed and must unfold before translocation. Unfolding probably involves removal of some nuclear proteins from the BR RNPs; however, many of the protein components of the RNP translocate through the NPC in association with the RNA (Daneholt, 1997). The extended BR RNP, in the form of a 25 nm diameter ribbon, traverses the entire length of the central channel. Indeed, BR RNPs can already have begun translation in the cytoplasm while their 3′ ends are still nuclear (Daneholt, 1997).

These export events tell us several important things about NPC translocation. First, the BR RNP extends beyond both ends of the transport channel during export, indicating that both ends of the channel can be open simultaneously. Second, multiple contacts between this single RNP and the NPC occur at the same time, likely indicating that RNP substrates are associated with multiple export receptors (Daneholt, 1997; Mattaj and Englmeier, 1998, for review). To maintain the integrity of the transport substrate, translocation of these receptors must be coordinated. There is a strong prediction that one export receptor, CRM1, should associate with the 5′ end of the BR RNP via the nuclear Capbinding complex, CBC (Izaurralde et al., 1995; Fornerod et al., 1997a). Together with CBC, CRM1 will be removed from the RNP on the cytoplasmic side of the NPC before the bulk of the BR RNP has even begun translocation (Visa et al., 1996). Translocation probably ceases when all receptors have been removed from the substrate, implying that the association of multiple receptors with a single mRNP substrate not only occurs but is necessary.

The distance from the tip of the cytoplasmic filaments to the terminal ring of the nuclear basket is roughly 200 nm. Receptors must make multiple contacts with nucleoporins as this distance is traversed. It is not yet known how many contacts and which nucleoporins are involved. Models of the translocation process have been proposed (Melchior et al., 1995; Radu et al., 1995; Görlich and Mattaj, 1996), but none are supported by strong evidence and therefore they will not be described.

Another point of ignorance concerns the source of energy for translocation. There is good but not definitive evidence that GTP hydrolysis by Ran is required for Importin-mediated import (Melchior et al., 1993; Moore and Blobel, 1993; Weis et al., 1996; for a critical discussion, see Mattaj and Englmeier, 1998). There is also evidence that at least some forms of nuclear export require nuclear RanGTP (to enable the formation of export receptor-substrate complexes; Figure 1) but may not depend on hydrolysis of the Ran-bound GTP (Izaurralde et al., 1997; Richards et al., 1997), suggesting that different forms of receptor-mediated translocation may depend upon diverse energy sources. Insight into this question may have to await the development of simpler translocation assays. Those currently available absolutely require the presence of an intact nucleus, making

it difficult to pinpoint where energy might be stored or what precise event accompanies hydrolysis of Ran-associated GTP.

Toward the Function of Individual Nucleoporins

Several approaches to defining the functions of single nucleoporins in translocation have been taken. The possibility of assembling NPCs in *Xenopus* egg extracts from which individual nucleoporins, or NPC subcomplexes, have been removed was already discussed. These studies can also be extended by analyzing the effect on transport of the injection of antibodies against individual NPC components. For example, it was found that nuclear assembly and NLS protein import were unaffected by either depletion of Nup98 from assembly extracts or by injection of antibodies directed against Nup98 into *Xenopus* oocytes (Powers et al., 1995; 1997). The export of multiple classes of RNA was, however, blocked by anti-Nup98 antibody injection (Powers et al., 1997).

The equivalent genetic approach is to analyze the effect of mutation or deletion of a specific nucleoporin. One study in mouse has implicated CAN/Nup214 in nucleocytoplasmic transport (van Deursen et al., 1996), and this approach has provided an abundance of information in yeast. Analysis of the effect of a nucleoporin mutant on a specific type of transport depends on having an appropriate assay. Methods were developed several years ago that enable detection of accumulated poly(A)-containing RNA in yeast cell nuclei. This is usually interpreted as a defect in mRNA export (Amberg et al., 1992; Kadowaki et al., 1992), but it should be borne in mind that alternative explanations for this phenotype are possible. Multiple yeast nucleoporin mutants exhibit nuclear poly(A) accumulation, and in several cases this phenotype was not accompanied by a detectable loss of nuclear protein import activity, suggesting a specific mRNA export defect (reviewed by Rout and Wente, 1994; Doye and Hurt, 1997).

Until recently, this interpretation was often problematic because assaying nuclear protein import required mRNA production, export, and translation under conditions in which the nucleoporin mutants were "semi-conditional." The development of an assay for NLS-protein import that does not require new translation of the reporter protein (Shulga et al., 1996) provides more definitive results and has already been used to confirm that mutation of two yeast proteins, Mex67p (Segref et al., 1997) and Gle1p/Rss1p (Del Priore et al., 1996; Murphy and Wente, 1996), indeed blocks poly(A) RNA export without affecting NLS protein import. Whether these two proteins are additional examples of nucleoporins that must be intact for mRNA transport to occur or, as suggested for Mex67p by the fact that it can be crosslinked to RNA in vivo, are more directly and specifically involved in mRNA export, remains to be established.

How far can the results of biochemical or genetic depletion studies be interpreted? One limitation is that it is very difficult to be sure that the primary lesion caused by a mutant nucleoporin is not a structural one. For example, mutating Nup170p/Nle3p has been shown to cause either an increase or a decrease in the efficiency with which other nucleoporins are incorporated into the NPC (Kenna et al., 1996). In addition, when comparing the effect of a specific nucleoporin mutation on NLS protein import and poly(A) RNA export, it is necessary to bear in mind that the latter substrate may well be 50–100 times the mass of the former, because mRNAs are coated with RNP proteins during their export. An apparently specific defect may therefore be a reflection of a structural defect serious enough to prevent passage of large but not small transport complexes. Nucleoporin-specific antibody inhibition studies could also suffer from the same problem, with the nonspecific steric effects of an NPC-bound antibody being more inhibitory to passage of a big transport complex.

One approach to answering these criticisms is to establish biochemical correlates of the observed phenotypic defects. Indeed, interactions between nucleoporins and Importin β family members have been found. The FG-containing repeat regions present in many nucleoporins interact in vitro or in the yeast two-hybrid assay with several members of the transport receptor family (Iovine et al., 1995; Radu et al., 1995; Rexach and Blobel, 1995; Aitchison et al., 1996; Fritz and Green, 1996; Stutz et al., 1996; Fornerod et al., 1997b; Neville et al., 1997; Rout et al., 1997). The functional relevance of these interactions has received support from experiments in which FG repeat–containing regions of some nucleoporins were either overexpressed or microinjected into cells and shown to cause nucleocytoplasmic transport defects (Iovine et al., 1995; Bastos et al., 1996; Stutz et al., 1996). However, a survey of the literature concerning receptor–FG repeat interactions reveals that there seems to be little specificity. In other words, receptor binding in vitro seems to be a generic property of many, perhaps even all, FG repeat–containing nucleoporins. This contrasts with the finding that the FG repeat region of yeast Nup116p cannot be replaced by substitution of the FG repeat regions from other nucleoporins in vivo (Iovine et al., 1995). Thus, we are left with a dilemma. Are the interactions seen in the in vitro assays meaningful, with specificity perhaps being dictated by the position of the nucleoporins on the NPC, or are they simply artefacts? Techniques like protein–protein crosslinking need to be applied to determine which interactions actually occur during docking and translocation. It would also be useful to develop methods that would allow identification of nucleoporins that are exposed on the aqueous surface of the NPC; for example, one method might involve using large water-soluble protein modification agents to map which of the many nucleoporins might contact transport complexes.

Rip1p and RanBP2/Nup358

We will end by describing two nucleoporins that are particularly interesting. Messenger RNAs encoding heat shock proteins (HS mRNAs) have to be made and exported to the cytoplasm after cells are exposed to heat shock or other elicitors of the stress response. Heat shock, however, results in the inhibition of bulk mRNA export (Saavedra et al., 1996). HS mRNAs are exported by an unusual mechanism that does not require normal mediators of mRNA export, including, remarkably, Ran and its effectors (Saavedra et al., 1996; 1997). Rip1p is

an FG repeat containing nucleoporin that is not essential for either growth in normal conditions or bulk mRNA export (Stutz et al., 1995). Rip1p is, however, required for HS mRNA export (Saavedra et al., 1997; Stutz et al., 1997). In relation to the previous discussion of the possible function of nucleoporin repeats, it is interesting that the FG repeat region of Rip1p, which has been shown to interact with various import and export receptors (Neville et al., 1997, and references therein), is not required for HS mRNA export. Rather, the unique C-terminal region of Rip1p is needed for this function. Further work will clarify if Rip1p makes specific interactions with receptors or adapters required for HS mRNA export, or whether it might rather protect the structural integrity of the NPC against the effects of heat shock and thus be necessary to allow passage of the HS mRNPs.

RanBP2/Nup358 has four RanGTP-binding domains, each of which resembles the cytoplasmic RanBP1 protein (Wu et al., 1995; Yokoyama et al., 1995). RanBP1 cooperates with the cytoplasmic RanGAP1 (GTPase-activating protein) to allow Ran to achieve maximal GTPase activity. RanBP1 and RanGAP1 can also cooperate to cause hydrolysis of GTP bound to Ran molecules associated with at least two transport receptors, Importin β and CAS (Floer et al., 1997; Kutay et al., 1997b; Lounsbury and Macara, 1997), and it is likely that this will hold true for many transport receptors. The four RanBP1-like domains of RanBP2 probably function similarly. RanBP2 also interacts directly with RanGAP1 or more precisely with a modified form of RanGAP1 to which a ubiquitin-like peptide has been conjugated (Matunis et al., 1996; Mahajan et al., 1997; Saitoh et al., 1997). Third, with the caveats mentioned above, RanBP2 interacts with transport receptors through its FG repeats.

Because RanBP2 is part of the cytoplasmic filaments of the NPC (Wu et al., 1995; Yokoyama et al., 1995), it provides a site on the periphery of the NPC where GTP hydrolysis will be highly favored. Two possible functions for RanBP2 have therefore been proposed. First, RanBP2 might be a site where RanGTP that arrives at the cytoplasmic side of the NPC, either on its own or in a complex with an exported transport receptor, would be preferentially hydrolyzed (Lounsbury and Macara, 1997). This could both allow rapid dissociation of export complexes after translocation and help prevent RanGTP-mediated dissociation of import complexes in the cytoplasm before their transport (Figure 1). In this model, RanBP2 would simply serve to increase the efficiency of the cytoplasmic RanBP1/RanGAP1 function by allowing a proportion of the exported RanGTP to be met and hydrolyzed at the cytoplasmic face of the NPC. Alternatively, it has been suggested that RanBP2 might be involved in coupling the energy derived from GTP hydrolysis by Ran to NPC translocation (Mahajan et al., 1997, and references therein). A problem with this idea is that RanBP2 is not conserved in *S. cerevisiae*. Given the presumably fundamental nature of coupling energy generation to translocation, one might expect factors required for this function to be conserved.

A region of RanBP2 resembles cyclophilin A, a peptidyl-prolyl *cis-trans* isomerase. In fact, in *Drosophila* photoreceptor cells, RanBP2 has been proposed to cause

isomerization of red/green opsin (Ferreira et al., 1996). The implication is that RanBP2 may be causing isomerization of prolines either in transported proteins or in components of the NPC itself.

Prospects

Despite the enormous ingenuity that has characterized the multidisciplinary effort to understand the structure and function of the NPC and despite the very real advances that are described in this review, all the important questions about how the NPC functions remain open. The identification of the components of the yeast NPC will be completed within the next few years. Beyond this, we believe that further and more exact topological mapping of individual nucleoporins will provide essential background information necessary to deduce function. With the recent identification of a family of nuclear import and export receptors, it is to be expected that methods to define which of the nucleoporin–receptor interactions are meaningful will be forthcoming. Understanding NPC function remains, quite literally, one of the biggest problems in cell biology.

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