

Dynamic Nuclear Pore Complexes: Life on the Edge

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The exchange of molecules between the nucleus and cytoplasm is mediated through nuclear pore complexes (NPCs) embedded in the nuclear envelope. Altering the interactions between transport receptors and their cargo has been shown to be a major regulatory mechanism to control traffic through NPCs. New evidence now suggests that NPC proteins play active roles in translocation, and that transport is also controlled by dynamic changes in NPC composition and architecture. This view of ever-changing NPCs necessitates the re-evaluation of current models of nuclear transport and how this process is regulated.

Introduction

The transport of molecules between the nucleus and the cytoplasm is necessary for the exchange of information within the eukaryotic cell. Historically, the nuclear pore complex (NPC) has been presented as a static channel through which dynamic transport receptor-cargo complexes traverse the nuclear envelope. However, recent evidence illustrates that the NPC is itself a dynamic, adaptive macromolecular complex whose components directly influence the status of cargo transport within the cell. This view is supported by new higher-resolution images of the NPC ultrastructure. In addition, genomic and proteomic advances have allowed large-scale direct manipulation of the NPC, providing new insights into the translocation mechanism. Nuclear transport can be controlled by alteration of NPC composition and by transient association of dynamic pore components. The NPC may undergo structural changes during different transport or cell cycle stages. There is also remarkable emerging evidence of dynamic coupling between NPCs and transcriptionally active gene loci. The focus of this review is to re-evaluate our current understanding of nucleocytoplasmic exchange and to envision the NPC as an ever-changing portal with the potential to regulate crucial cellular functions both locally and globally.

Nucleocytoplasmic Transport

At the very edge of the nucleus, a pathway for movement of molecules across the nuclear envelope is provided by the aqueous channels within the NPCs. In the past, the function of the NPC has been relegated to constitutive maintenance of the nuclear permeability barrier. Molecules smaller than 25–40 kDa can passively diffuse across the NPC; however, highly efficient localization and all macromolecular transport events require facilitated, energy-dependent transport (reviewed in Fried and Kutay, 2003).

Such active transport is dependent on either an intrinsic capacity to interact directly with the NPC or association with different types of transport receptors. The largest class of transport receptors is designated karyopherins, or Kaps, and includes different importins, exportins, and transportins that may be specialized for import or export. A very well characterized Kap, importin β (Kap95 in yeast), binds to import cargo through an adaptor protein, importin α (Kap60 in yeast), and has been shown to interact with several NPC proteins (reviewed in Pemberton and Paschal, 2005). The directionality of Kap-mediated transport is exquisitely controlled by the small GTPase Ran, whose nucleotide-bound state dictates cargo binding and release in a compartmentalized manner (reviewed in Fried and Kutay, 2003). Overall, active nuclear transport is a highly orchestrated, rapid, and efficient process. Estimates from both in vitro transport rate studies and single-molecule imaging analysis suggest an import rate capacity of 1000 molecules per second per NPC, requiring that at least 10 molecules must traverse a given NPC simultaneously (Ribbeck and Gorlich, 2001; Yang et al., 2004). Thus, the NPC is capable of orchestrating multiple concurrent transport events.

The NPC is a huge macromolecular assembly with a calculated mass of 40 and 60 MDa in yeast and vertebrates, respectively (Cronshaw et al., 2002; Rout et al., 2000). Despite the difference in size, the basic architecture of the NPC is conserved between yeast and metazoa. The overall structure of the NPC can be superficially divided into three basic elements: the nuclear basket, the central core, and the cytoplasmic fibrils. Recently, state of the art cryoelectron tomography with three-dimensional reconstruction has been used to present the highest-resolution structure (approximately 9 nm) of the NPC to date (Beck et al., 2004). This structure shows that the cytoplasmic filaments adopt a highly “kinked”

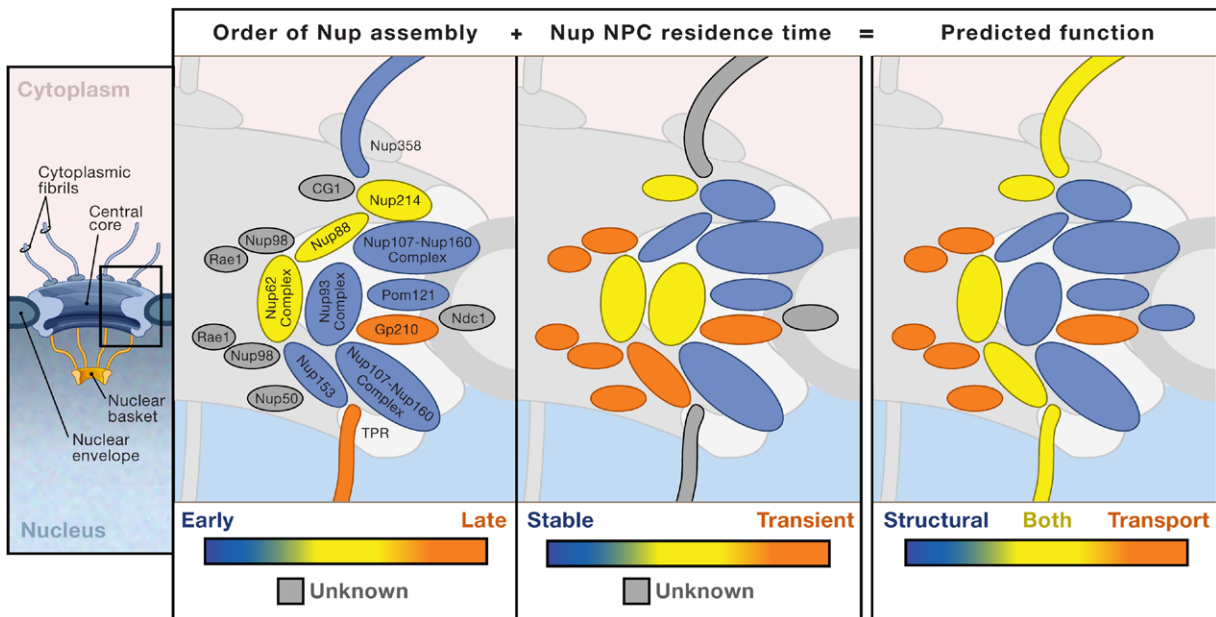


Figure 1. NPC Assembly and Nup Dynamics Are Correlated with Proposed Function within the NPC

A cross-section of an NPC is shown, with the central region magnified in the right three panels. General structural features are based on data reviewed in Schwartz (2005) and Table 1. The order of Nup assembly (late to early) into NPCs following mitosis (Hetzer et al., 2005, and references therein) and the relative Nup NPC residence time or shuttling activity (stable to transient) (Griffis et al., 2002; Pritchard et al., 1999; Rabut et al., 2004) are illustrated. The predicted function (structural to transport) for each Nup or subcomplex is also shown. Common colors between the three figures in each structure indicate correlations in assembly, dynamics, and function. Those that are early in assembly and stable in residence time are likely structural in function. In contrast, those that are late in assembly and transient in residence time are likely directly involved in transport.

structure, yielding a length of approximately 35 nm. A similar bent conformation is observed for the nuclear basket filaments, indicating that a transport complex travels 120 nm instead of the 200 nm distance previously predicted (Stoffler et al., 2003).

NPCs are composed of approximately 30 proteins, collectively called nucleoporins (Nups) (summarized in Cronshaw et al., 2002; Rout et al., 2000). These Nups have long been classified into groups based on the presence or absence of primary sequence motifs. A convergence of recent direct structural analysis and structural modeling studies have gone one step further and now reveal three striking protein structure fold types that might constitute more than 85% of the complex (Devos et al., 2004, 2006; Rout et al., 2000). At the pore membrane, at least three proteins with transmembrane helices, the first structural fold, have been functionally and structurally linked to the NPC (Mansfeld et al., 2006; Schwartz, 2005). These membrane-anchored proteins are presumably directly connected to a second structural module composed of Nups of two specific fold types, the β propeller and α solenoid. Finally, a third distinct group of peripheral Nups comprises at least a third of the total NPC mass and contains the FG Nups.

The FG Nups are so named for discrete domains harboring repetitive stretches of Phe-Gly residues separated by polar spacers of variable length (reviewed in Rout and Wentz, 1994). The FG domains are directly implicated

in active nuclear transport (see below). Interestingly, the FG domains themselves are thought to be natively unfolded (Denning et al., 2003). This would allow them to potentially have multiple topological locations in the NPC based on their site of anchoring by their respective non-FG domains. The non-FG domains of the FG Nups have predicted coiled-coil, β propeller, or unique β sandwich structures (Nup98 fold) (Hodel et al., 2002; Weirich et al., 2004). These might assemble on to the structural scaffold provided by the β propeller- α solenoid core (Devos et al., 2004, 2006, and references therein). In effect, the unfolded FG domains may coat the scaffold.

The prevalence of the β propeller and α solenoid domains was surprising, and a modular view of the NPC at the edge of the nuclear envelope has emerged (reviewed in Schwartz, 2005 and Figure 1). Moreover, the unfolded nature of the FG domains is a key tenet of nuclear transport models. Finally, intriguing structural similarities between these β propeller and α solenoid Nups and proteins in the Golgi-mediated transport system suggest common ancestral origins in evolution.

The NPC Is Dynamic

As the static images in Figure 1 suggest, NPC structure could easily be viewed as being of a defined composition with each Nup at permanent locales. A wealth of studies has changed this view at multiple levels. In fact, the NPCs may undergo several types of dynamic modifications. This

Table 1. Summary of Vertebrate and Yeast Nups or NPC Subcomplexes

	Vertebrate Nups and Nup Subcomplexes ^a	Yeast Nups and Nup Subcomplexes ^a	Order of Assembly ^b	Dynamics ^c	Structural Folds ^d	Predicted Function(s)
Cytoplasmic Face	Nup358	—	Early	ND	FG	Structural and transport
	Nup214	Nup159	Middle	Stable	Bp, CC, FG	Structural
	CG1	Nup42	ND	Intermediate	FG	Structural and transport
	Nup88	Nup82	Middle	Stable	Bp, CC	Structural
Symmetrically Distributed	Nup62 complex	Nsp1 complex	Middle	Intermediate	FG, CC	Structural and transport
	Nup107-160 complex	Nup84 complex	Early	Stable	Bp, As	Scaffold
	Nup93 complex	Nic96 complex	Early	Intermediate	Bp, As, FG	Structural and transport
	Rae1/Gle2	Gle2	ND	Dynamic	Bp	Transport
	Nup98	Nup100, Nup116 and Nup145N	ND	Dynamic	FG, Nup98 fold	Transport
Transmembrane	Pom121	—	Early	Stable	TMH, FG	Structural
	gp210	Pom152	Late	Dynamic	TMH	Transport
	Ndc1	Ndc1	ND	ND	TMH	Structural
	—	Pom34	ND	ND	TMH	Structural
Nuclear Face	Nup50/Npap60	Nup2	ND	Dynamic	FG	Transport
	Nup153	Nup1	Early	Dynamic	FG	Structural and transport
	—	Nup60	ND	Dynamic	FG	Transport
	TPR	Mlp1 and 2	Late	ND	CC	Structural and transport

Bp, β propeller; As, α solenoid; FG, FG repeat domains; CC, coiled coil; ND, not determined.

^aThe Nup components of the vertebrate (v) and yeast (y) NPC complexes are as described (Hetzer et al., 2005; Loiodice et al., 2004; Mansfeld et al., 2006; Schwartz, 2005), and subcomplex members are as follows:

vNup62 complex: Nup62, Nup58, Nup54, Nup45; yNsp1 complex: Nsp1, Nup57, Nup49; vNup107-160 complex: Nup160, Nup133, Nup107, Nup96, Nup75/85, Nup43, Nup37, Sec13, Seh1, ALADIN; yNup84 complex: Nup145C, Nup133, Nup120, Nup85, Nup84, Sec13, Seh1; vNup93 complex: Nup205, Nup188, Nup155, Nup93, Nup35/53; yNic96 complex: Nic96, Nup192, Nup188, Nup170, Nup157, Nup59, Nup53.

^bReviewed in Hetzer et al. (2005).

^cCompiled from Griffis et al. (2002); Lindsay et al. (2002); Pritchard et al. (1999); Rabut et al. (2004).

^dDevos et al. (2006) and references therein.

includes conformational changes in the structure during transport, compositional alterations due to transient and regulated association of different Nups, and more global structural transitions during the cell cycle.

Early suggestions of the NPC structure as a dynamic entity came from EM studies in which the central channel was observed in both a closed and open state, reminiscent of an iris (Akey, 1995). The closed state of the NPC was later described as a central plug, most likely composed of transporting cargo, and not representative of structural alterations (Stoffler et al., 2003). However, major structural changes, albeit of a different nature, might be correlated with transport status of the NPC. Using intact nuclei and cryoelectron tomography, two different NPC conformational classes have been described (Beck et al., 2004). A subset of NPCs in these samples have cytoplasmic filaments bent toward the central channel, potentially inter-

acting directly with cargo during transport. The other NPC class is characterized by extended, disordered cytoplasmic filaments. These two structurally distinct NPC conformations may represent different stages of nuclear transport, providing “snapshots” of active translocation through the pore. These structures might also reflect differences in the composition of Nups from one NPC to another based on differential association of dynamic Nups. Although the nuclei examined are globally competent for transport, the method used to purify them might have caused the inactivation of some of the NPCs. Therefore, caution is required before assuming that both conformations represent functional NPCs engaged in transport.

Independent EM evidence of potential changes in the NPC structure in vivo has come from reports illustrating altered distributions of individual Nup domains within the NPC under different transport conditions (Fahrenkrog et

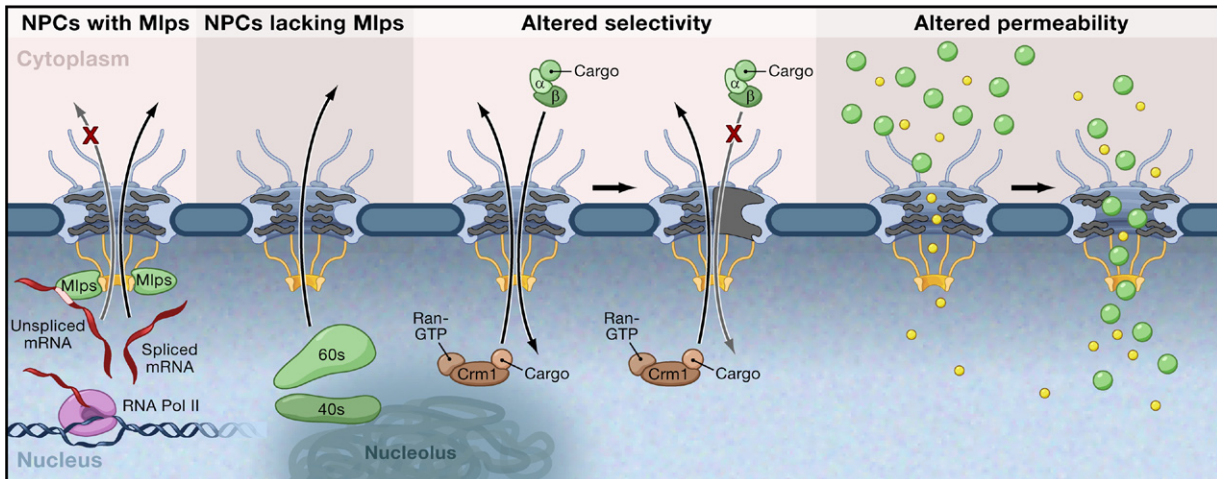


Figure 2. NPCs Vary in Composition and Transport Activity Between and Within Cells

The Mlps (yeast orthologs of vertebrate TPR) are associated with a subset of NPCs, possibly to facilitate their function in mRNA export quality control by active retention of unspliced mRNA (Galy et al., 2004). However, in budding yeast, Mlps are excluded from NPCs adjacent to the nucleolus (Galy et al., 2004). This may facilitate ribosome export through an uncharacterized mechanism. By changes in the FG Nups (gray filaments), NPCs might have altered selectivity; e.g., classical nuclear import is inhibited in vertebrate cells infected by poliovirus, whereas nuclear export is not perturbed (reviewed in Gustin, 2003). The permeability barrier maintained by the NPC can also change; e.g., during closed mitosis in *A. nidulans*, the diffusion size limit increases (De Souza et al., 2004). α , importin α ; β , importin β .

al., 2002; Paulillo et al., 2005). For example, the C-terminal FG domain of Nup214 is detected at the nuclear face of the NPC within 30 min postinjection of poly(A+) RNA into *Xenopus* oocyte nuclei, whereas the N-terminal region appears stably associated with the cytoplasmic fibrils. If fully extended and unfolded, the almost 300 nm predicted length of the Nup214 FG domain is certainly adequate to span the 120 nm central core distance (Beck et al., 2004; Paulillo et al., 2005). However, it is not known if such changes are a direct or an indirect consequence of transport. It is also not clear whether domain movement or cytoplasmic fibril dynamics are requirements for transport. Other studies have clearly shown that the cytoplasmic fibrils are dispensable for in vitro steady-state importin β nuclear import (Walther et al., 2002) and that all the FG domains in the cytoplasmic fibrils and nuclear basket Nups can be removed without significant impact on Kap95 (yeast importin β) import (Strawn et al., 2004). Thus, these conformational changes may indeed occur and increase transport efficiency, but they are likely not strictly required for NPC translocation.

More direct evidence for structural dynamics in NPC composition comes from a large-scale analysis of the NPC residence times for individual Nups. Using a fluorescence-based strategy in vertebrate cells (Rabut et al., 2004), some NPC components were found to have very low turnover rates and are virtually static residents of the NPC; others exhibit transient associations with the NPC, indicative of rapid dynamics (Figure 1 and Table 1). Consistent with this study, some of the Nups described as having a high turnover also have documented shuttling activity, that is, the ability to move between the nucleus and cytoplasm (Griffis et al., 2002; Lindsay et al., 2002).

Thus, some Nups spend their entire “life” at the NPC, whereas others experience multiple subcellular environments. It is also intriguing that the Nups with dynamic NPC association are also structurally correlated with common NPC modules. Future studies will reveal if individual Nup dynamics are critical for NPC function and whether this is a general feature of the NPC in all cell types.

Novel Nup Dynamics in Mitosis

NPC dynamics are clearly critical during cell cycle transitions (reviewed in Hetzer et al., 2005). The entry into mitosis in higher eukaryotic cells is coincident with nuclear envelope breakdown and the reversible disassembly of the NPC during mitosis. Mitotic Nups are presumably maintained in different types of subcomplex modules that serve as building blocks for NPCs during telophase. The mechanism of NPC reassembly at the end of mitosis has been studied in depth, and an emerging picture of stepwise recruitment is in place (summarized in Figure 1, Table 1, and Hetzer et al., 2005), and global NPC dynamics during mitosis might be based on regulated posttranslational modifications.

An elegant study by Osami and colleagues has highlighted novel mitotic Nup dynamics that impact the structure and function of NPCs (De Souza et al., 2004). Although the slime mold *Aspergillus* undergoes a closed mitosis in which the nuclear envelope remains intact and the majority of the Nup components appear to stay associated with the NPC during mitosis, a subset of Nups, including Gle2/Rae1 and Nup98, are dispersed. These authors show that phosphorylation of Gle2/Rae1 and Nup98 by mitotic kinases results in this altered NPC composition. Surprisingly, this change in composition is coincident with altered NPC permeability (Figure 2). This indi-

cates that at least some fungal species have ways to alter the nuclear permeability barrier without an open mitosis and complete NPC disassembly. Alterations of the NPC during the cell cycle may not be exclusive to this fungal species, as partial disassembly via release of peripheral Nups has also been noted for starfish oocytes prior to nuclear envelope breakdown (Lenart et al., 2003).

In contrast, the budding yeast *Saccharomyces cerevisiae* NPC has not been reported to undergo any level of mitotic NPC disassembly. However, one study has demonstrated that specific transport pathways are altered during the budding yeast cell cycle (Makhnevych et al., 2003). This occurs by molecular rearrangements of Nup associations within the NPC. During interphase, Nup53 is bound to Nup170; however, during mitosis, Nup53 is no longer bound to Nup170 and is instead associated with Nic96. This rearrangement, likely a result of Nup53 phosphorylation, results in exposure of a high-affinity Kap121 binding site on Nup53 and inhibition of Kap121-dependent transport. How this alteration in Kap121 transport impacts mitotic progress remains unresolved. Taken together, these recent studies have demonstrated that structural and compositional changes in the NPC are mechanisms by which transport flux and individual pathways can be regulated.

Several NPC components whose function in transport is documented have also been tied to cellular activities distinct from their roles at the nuclear envelope. A wealth of recent data has documented the localization of Nups at kinetochores during mitosis. Multiple members of the vertebrate Nup107–160 complex show this kinetochore association, as does Nup358 (reviewed in Chan et al., 2005). The Gle2/Rae1-Nup98 complex serves as an excellent example to illustrate the complex roles for Nups in mitotic mechanisms. Using a *Xenopus* egg extract system for mitotic spindle formation, Gle2/Rae1 was identified as a necessary component for promotion of microtubule assembly (Blower et al., 2005). *GLE2/RAE1* depletion by RNAi in HeLa cells resulted in clear defects in spindle assembly, illustrating that Gle2/Rae1 has a role at this stage of mitosis. In a contrasting report, however, the van Deursen lab identified the Gle2/Rae1-Nup98 complex as an inhibitor of the anaphase promoting complex (APC) in a mouse model system (Jeganathan et al., 2005). These results suggested that Gle2/Rae1-Nup98 temporally controls the onset of anaphase by preventing untimely securin degradation mediated by the APC. Interestingly, the latter study saw no defect in spindle assembly. Therefore, the precise role of the Gle2/Rae1-Nup98 complex in mitosis is far from resolved.

Are All NPCs the Same?

Several nuclear transport receptors exhibit differential requirements in multicellular organisms. For example, there are Kap family members with well documented changes in expression during development and, in some cases, tissue-specific expression of variants within an organism (reviewed in Poon and Jans, 2005). Such com-

positional variance was not generally thought to be the case for the Nups themselves. Several reports now suggest that the composition of the NPC can indeed be varied in a manner that might regulate transport. A notable recent example is that described above for the *Aspergillus* NPC changes during the cell cycle. Other mechanisms are highlighted here by which each NPC could be considered structurally and functionally unique. With NPCs having the capacity to change or be altered to modulate transport pathways, it directly follows that the composition of all NPCs may vary from tissue to tissue in a multicellular organism, between developmental stages, and within the nuclear envelope of a single cell (Figure 2).

One example of a NPC protein that exhibits tissue-specific expression levels is gp210, a transmembrane spanning protein with dynamic NPC association (Rabut et al., 2004). In mouse embryos, gp210 levels vary widely across different tissues compared to Nup62 (Olsson et al., 2004). In another case, mutants of *members only* in *Drosophila*, a homolog of vertebrate Nup88, exhibit cell-type specific phenotypes that are linked to specific import defects (Uv et al., 2000). Given that NPC composition varies among differentiated cell types, it is not surprising that NPCs are not the same during different stages of cell development. Early evidence for such compositional changes in the NPC arose from studies of Nup50 (Npap60) in rat spermatogenesis (Fan et al., 1997). Nup50 is 10–20 times more abundant in testis than in other rat tissues and exhibits strikingly different cellular localization patterns depending on the sperm maturation stage. For example, Nup50 is associated with the NPC in spermatocytes but is nucleoplasmic in spermatids, whereas other NPC components remain associated with the nuclear envelope. However, a role for the dynamic Nup50 localization patterns in changing transport pathways has not been determined. In contrast, a more recent study has proposed a role for a splice variant of Nup358, BS-63 (Cai et al., 2002). BS-63 is found only in testis and associates with the NPC in spermatids. Interestingly, a yeast two-hybrid study revealed direct interaction between BS-63 and a germ cell-specific transcription factor, AF10 (Cai et al., 2002). AF10 may be imported through direct interaction with the NPC through BS-63, similar to β catenin. As previously suggested, BS-63 may act as a developmental stage-specific docking site for specific transport receptor complexes (Cai et al., 2002).

Evidence of compositional variation of the NPC certainly challenges the dogma that all NPCs within the same cell are identical. Mlp1 and Mlp2 (homologous to the vertebrate TPR) are highly similar coiled-coil proteins associated with the nuclear basket of the NPC in yeast. Strikingly, these proteins are not equally distributed around the nuclear envelope rim. Instead, they are excluded from regions adjacent to the nucleolus and are found only near NPCs associated with presumably active chromatin (Galy et al., 2004). Thus, Mlp proteins may be components of only a subset of NPCs, indicating that NPCs can be dis-

tinct in composition within a given cell. More importantly, this compositional difference has functional consequences. The Mlps have been linked to multiple aspects of nuclear physiology, including maintenance of mRNA export fidelity (Galy et al., 2004). These results are indicative of a coupling of upstream events, such as mRNA biogenesis, with nuclear transport through the activity of specific Nups and NPC-associated proteins (see below). As compared to NPCs without Mlps, the Mlp-associated NPCs might be committed to specific transport functions such as quality control in mRNA export through active mRNA retention (Figure 2). Although not yet documented experimentally, the non-Mlp NPCs localized near the nucleolus might be specialized for transport events required in ribosome biogenesis.

Why Have a Dynamic Pore?

The precise role of an actively adaptive pore in transport processes is not well characterized. However, the simplest scenario positions the NPC as a regulator of nucleocytoplasmic exchange. This hypothesis stands in clear contrast to the current field of transport, which places the vast majority of regulation on modulation of the Kap-cargo interaction (reviewed in Poon and Jans, 2005). Mechanisms for regulation of Kap-cargo interactions include both masking of a localization signal within the cargo and posttranslational modification of sites near or within the localization signal. These alterations affect the ability of a Kap to recognize its cargo or modulate the affinity of the Kap-cargo interaction. These changes also provide regulation of a specific nuclear transport pathway, a critical feature for any regulatory mechanism. How then would dynamic changes in the NPC modulate a precise transport event rather than globally affecting transport? One possibility is that multiple, nonequivalent transport pathways exist within a given pore complex.

The existence of multiple pathways is supported by biophysical studies illustrating rates of import incompatible with single pathway models (Yang et al., 2004; Kubitscheck et al., 2005). There are an estimated 128 FG domains harboring thousands of total FG repeats within a given NPC in yeast (Rout et al., 2000). The FG repeats provide multiple, low-affinity NPC binding sites for transport receptor complexes during active transport (Ribbeck and Gorlich, 2001). Early evidence for distinct FG pathways came from the finding that a budding yeast mutant defective in the function of one FG Nup (*nsp1-S5*) only impaired distinct import pathways (Nehrbass et al., 1993). To fully investigate the contributions of specific FG domains within the yeast NPC, large-scale deletion analysis was performed across the entire complement of yeast Nups (Strawn et al., 2004). This work generated NPCs with minimal repertoires of FG domains and pinpointed the functionally important FG domains. The asymmetrically positioned FG domains in the peripheral NPC fibril structures are not essential. Perhaps more interesting, analysis of the Kap transport pathways in the minimal FG mutants revealed that different Kaps require different subsets of FG domains. This

in vivo data correlates with independent studies showing preferential in vitro binding of different Kaps to various FG Nups (Aitchison et al., 1996; Allen et al., 2001). Therefore, functionally independent routes through the NPC indeed exist, each potentially using the same biochemical mechanism of translocation (see below) but through different binding sites in the pore. The presence of such multiple pathways would allow regulation of specific transport pathways at the level of the NPC.

Additional support for such a multiple transport pathway model comes from studies of viral inhibition of nuclear transport (reviewed in Gustin, 2003). Members of the picornavirus family (which includes poliovirus and rhinovirus) specifically target two FG Nups, Nup153 and Nup62, for degradation and effectively inhibit classical nuclear import without affecting export (Figure 2). It is speculated that viruses inhibit protein import to evade host immune responses while maintaining other cellular activities necessary for viral proliferation (Gustin, 2003). Selective degradation of specific Nups is also a characteristic of cellular defense mechanisms. Interestingly, apoptosis globally inhibits nuclear transport by degrading both peripherally located Nups (the FG Nups Nup358, Nup214, and Nup153) as well two Nups within the NPC core without altering the overall NPC structure (Patre et al., 2006). These reports support a model whereby multiple pathways through the NPC are controlled by distinct Nup components and overall NPC composition.

A Unified Model for Translocation through the NPC?

Several models have been proposed in recent years speculating on the mechanism for facilitated movement through the NPC. These include the virtual gating model, the affinity gradient model, the selective phase partition model, and the oily spaghetti model (summarized in Fried and Kutay, 2003). A unifying feature of these models is that each invokes some type of facilitated diffusion controlled by association and disassociation of transport receptors with FG Nups. FG Nups have also been thought critical for maintenance of the NPC permeability barrier (Ribbeck and Gorlich, 2001; Rout et al., 2000). Results again stemming from genetic analysis of NPC function in yeast have impacted the models for NPC translocation. First, the FG domains present at the asymmetric NPC peripheral structures are not strictly required, and there is no preference for FG domain type at the fibril structures (Strawn et al., 2004; Zeitler and Weis, 2004). This means that translocation is not driven by sequential transport receptor binding to different FG Nups with progressively increasing FG affinity from one NPC face to the other. In addition, the peripheral FG domains are not essential for trapping at or recruiting transport receptors to the NPC (although they may contribute to transport efficiency for some pathways). Second, nearly half of the total FG mass of the NPC can be deleted without affecting cell viability, and there is no correlation between the amount of FG mass deleted and the impact on active transport. Moreover, diffusive permeability measurements in sev-

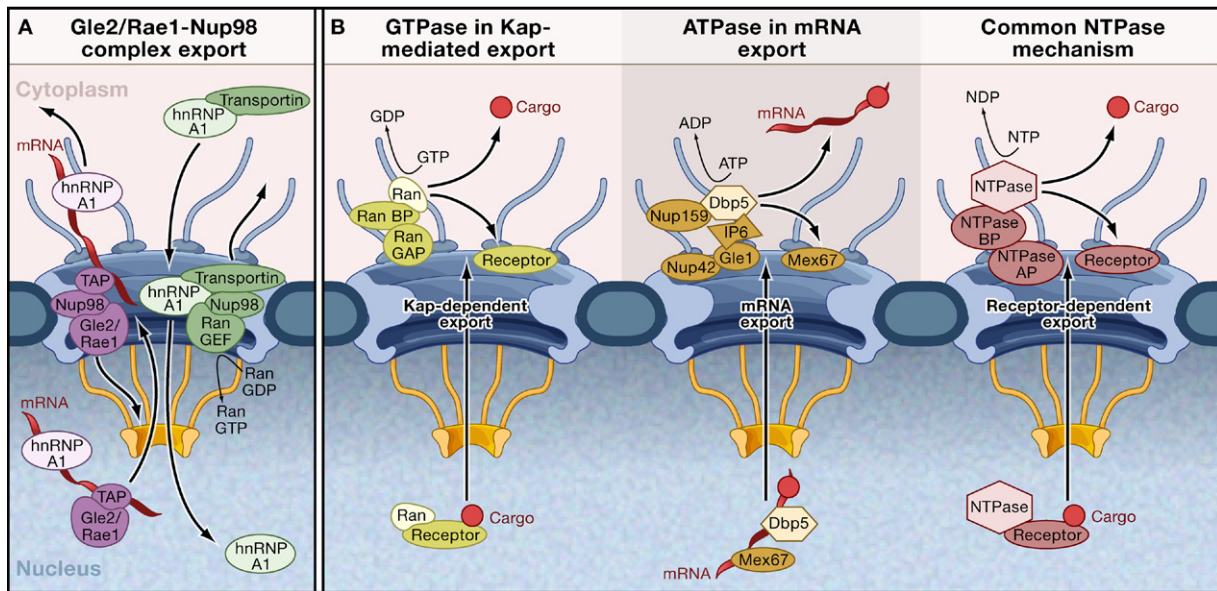


Figure 3. Individual Nups and NPC-Associated Factors Actively Facilitate Nuclear Transport

(A) The Gle2/Rae1-Nup98 complex is involved in targeting the mRNA export receptor, TAP/NXF1, to the NPC during mRNA export (Blevins et al., 2003) as well as promoting recycling of hnRNPs by affecting nuclear import (Fontoura et al., 2000).

(B) Both Ran and Dbp5, drivers of protein or mRNA export respectively, are targeted to the NPC cytoplasmic face and spatially activated by NTPase activating factors (AP). On the left, the role of Ran in nuclear export is illustrated with the respective binding protein (BP) Nup358 and GTPase activating protein (RanGAP) indicated (Fried and Kutay, 2003). SUMOylation is required for RanGAP association (Matunis et al., 1998). In the middle, Dbp5 and its putative function in mRNA export is shown. Docking of Dbp5 at yeast Nup159 (vertebrate Nup214) results in juxtaposition with the NPC-associated protein, Gle1 (Hodge et al., 1999; Schmitt et al., 1999). Association of IP_6 with Dbp5/Gle1 results in activation of Dbp5 ATPase activity (A.R. Alcázar-Román, E.J.T., S. Guo, and S.R.W., unpublished data). In the right merged panel, the common NTPase binding and activation mechanism in both export pathways is illustrated.

eral of the minimal FG NPCs did not reveal any defects (Strawn et al., 2004). Other studies have suggested that non-FG Nups in the NPC core structure are required for maintenance of the permeability barrier (Galy et al., 2003; Shulga et al., 2000). These findings indicate that the FG domains do not merely form a static permeability barrier and are, instead, involved in specific pathways. Indeed, the NPC structures required for active facilitated translocation might be physically distinct and not coupled to those required for forming the permeability barrier.

Whereas some FG domains are critical for given transport receptor pathways, it is possible that the sheer bulk of FG mass in the NPC has been maintained in the NPC to allow for high transport efficiency. As a result of these *in vivo* studies and biophysical measurements of NPC residence times for model transport substrates, a new model has emerged, termed reduction of dimensionality (Peters, 2005). This model proposes that FG domains line the inner surface of the NPC, whereas the polar spacers separating the FG domains form outstretched loops within the NPC interior. This topological arrangement of FG domains would restrict Kap-cargo movement in only two dimensions, facilitating rapid transport by restricting random movement within the pore. Certainly, FG bulk mass would not be strictly required as long as enough were present to provide an appropriate binding surface. Presentation of a model that includes recent findings of

NPC dynamics is a daunting challenge. Definitive resolution of the translocation mechanism will require further careful manipulation of the number and location of FG Nup binding sites in discrete NPC substructures.

Nups as Active Participants in Transport

Evidence presented thus far demonstrates that the NPC is a dynamic macromolecular complex, which can specifically regulate transport pathways. Although the NPC is not a static portal, the role of the NPC in transport could still be passive. However, several studies have now characterized specific Nups that function as direct transport facilitators and play an active role in transport. One example involves the role of Nup98, a dynamic FG Nup, in facilitating transport. First, Nup98 associates with RCC1 (Fontoura et al., 2000), the Ran guanine exchange factor (RanGEF) that promotes disassembly of Kap-cargo import complexes in the nucleus by catalyzing the exchange of GDP for GTP by Ran. Second, Nup98 also interacts with transportin (Fontoura et al., 2000), the Kap which recognizes an M9 import sequence in hnRNP A1 (Pemberton and Paschal, 2005). Interaction between transportin and Nup98 is inhibited by the presence of the M9 domain, suggesting that Nup98 may actively displace cargo from transportin receptor complexes at the NPC (Figure 3A). Indeed, Nup98 harbors a competing M9-like sequence. A possible model for the role of

these interactions in nucleocytoplasmic transport would include initial binding of transportin to Nup98 followed by release of hnRNP A1. Localized generation of RanGTP at Nup98 by RCC1 would subsequently promote release of transportin from Nup98, facilitating recycling of this receptor back into the cytoplasm for future transport events. This putative mechanism may facilitate efficient disassembly of receptor complexes but may also aid rapid movement of cargo across the nuclear envelope by removing the need for receptor translocation across the entire length of the nuclear pore. However, as the precise cellular location of the Nup98 interaction with RCC1 has not been identified, it is also possible that it occurs within the nucleus itself rather than at the NPC.

Recent studies have identified other specific motifs in distinct Nups that broaden this paradigm for Nup-Kap binding in a mechanism for releasing cargo. Nup50 (Npap60) has a unique Kap binding site that interferes with Kap-cargo interactions (Gilchrist et al., 2002; Matsuura and Stewart, 2005). In fact, Nup50 (yeast Nup2) uses a unique domain to actively displace importin α -bound nuclear localization sequences. This activity may promote efficient receptor-cargo complex disassembly upon import into the nucleus. Interestingly, importin α has two Nup50 binding sites, one of which overlaps with the Cse1 binding site (Matsuura and Stewart, 2004), the cognate export receptor for importin α . These overlapping sites have led to the proposal that Nup50 may use these unique binding properties to couple nuclear import complex disassembly with rapid recycling of import receptors back to the cytoplasm (Matsuura and Stewart, 2005). Such Nup-mediated receptor complex dissociation and release may be a critical component of transport efficiency.

Another example of direct involvement of Nups in nucleocytoplasmic exchange is again provided by the Gle2/Rae1-Nup98 complex. Recently, the mRNA export receptor TAP/NXF1 was shown to bind to both Gle2/Rae1 and Nup98 individually (Blevins et al., 2003). In fact, Gle2/Rae1 and TAP/NXF1 associate with Nup98 at different binding sites to form a stable, ternary complex (see Figure 3A). Interaction between Nup98 and Gle2/Rae1 was, however, mutually exclusive with association between TAP/NXF1 and Gle2/Rae1. Therefore, a mechanism can be envisioned whereby Gle2/Rae1 may actively deliver TAP/NXF1 to the NPC and promote "hand-off" to other Nups, facilitating rapid mRNA export (Figure 3A).

The fact that the Gle2/Rae1-Nup98 complex functions in both the export of mRNA and import (recycling) of mRNA export factors into the nucleus illustrates that this complex is an active player in the gene expression pathway. Why have one complex involved in two fundamental transport activities that are both required for mRNA export? This coupling of transport processes to a specific protein complex would enable rapid and efficient control of gene expression. In fact, the vesicular stomatitis virus (VSV) exploits this regulatory switch. VSV is a minus strand virus that upon infection specifically

inhibits host cell mRNA export through a virally encoded protein called M (Her et al., 1997). Gle2/Rae1 bridges the interaction between M protein and Nup98, leading to the conclusion that inhibition of the Gle2/Rae1-Nup98 complex is the means by which the M protein blocks mRNA export (Faria et al., 2005; von Kobbe et al., 2000). In further support of this model, increased Gle2 levels fully restore host cell mRNA export. Interestingly, both Gle2/Rae1 and Nup98 are upregulated during the cellular antiviral response by interferon γ (Faria et al., 2005). Therefore, cells have a mechanism in place to "titrate" the viral M protein and restore cellular functions by exploiting the same molecular switch within the NPC. Overall, it is clear that viruses use specific components within the NPC to alter nuclear transport.

Nup Coupling Sites for the Direction of Transport and Cargo Modification

Whereas Nups can actively function in transport through both dynamic association with the NPC and direct interactions with transport receptors (e.g., Kaps or TAP/NXF1), reports of Nups acting as docking sites for other cellular transport factors provide further evidence for active roles of Nups in transport. This docking scenario is best illustrated by Nup358. The vertebrate Nup358, a component of the cytoplasmic fibrils, functions as a molecular "Grand Central Station" for disassembly of transport complexes and recycling of transport factors. Nup358/RanBP2 harbors four Ran binding domains and a stable interaction site for a SUMOylated Ran GTPase-activating factor, RanGAP1 (Matunis et al., 1998). SUMO modification has been shown to influence protein-protein interactions (reviewed in Hay, 2005). Strikingly, Nup358 functions directly in SUMO modification of RanGAP1 along with the SUMO E2 ligase, Ubc9, by acting as an E3 ligase to stabilize a SUMO-modified RanGAP1 at the cytoplasmic fibrils (Pichler et al., 2002). As localization of RanGAP to the cytoplasm is a key determinant of directionality (reviewed in Fried and Kutay, 2003), it is clear that Nup358 is a critical component of nuclear transport.

Heterogeneous nuclear ribonucleoproteins (hnRNPs) may also be substrates for this SUMOylation complex, and SUMO modification of at least two hnRNPs (hnRNP C and hnRNP M) reduces their affinity for RNA (Vassileva and Matunis, 2004). hnRNP C proteins have been characterized as non-shuttling mRNA binding proteins (Pinol-Roma and Dreyfuss, 1992), and SUMOylation may aid in rapid recycling of SUMO-modified hnRNPs back into the nucleus and prevent their free diffusion into the cytoplasm. The fact that SENP2 (a SUMO protease in vertebrates) and Ulp1 (a SUMO deconjugating enzyme in yeast) have been structurally and functionally connected to the NPC is intriguing (Panse et al., 2003; Zhang et al., 2002). One group has shown connections between Ulp1 and the nuclear-faced Mlps and Nup60 in yeast, and others have Ulp1 anchored by Nup-associated Kaps (Panse et al., 2003; Zhao et al., 2004). This may allow for

an elegant cycle of SUMOylation of hnRNPs, or other transport cargo, in the cytoplasm, followed by rapid removal of the SUMO modification in the nucleus. However, Ubc9 has also been localized to the nuclear basket, most likely via Nup153 (Zhang et al., 2002). Therefore, the precise mechanism of SUMO-mediated recycling of hnRNPs remains undetermined.

In addition to a role for SUMO modification in triggering hnRNP release from mRNA, critical protein remodeling events in mRNA export are believed to be catalyzed at the NPC by the essential DEAD-box helicase protein, Dbp5. Like Ubc9, Dbp5 is also specifically localized at the cytoplasmic face of the NPC by association with a peripheral Nup, namely Nup214 (Nup159 in yeast) (Hodge et al., 1999; Schmitt et al., 1999). Using budding yeast as a model system, two independent laboratories have provided compelling evidence for highly localized activation of Dbp5 in mRNA export. Whereas recombinant Dbp5 has a very low intrinsic ATPase activity, association with Gle1, an essential NPC-associated mRNA export factor, activates Dbp5 ATPase activity in a manner that is stimulated by soluble inositol hexakisphosphate (IP₆) (A.R. Alcázar-Román, E.J.T., S. Guo, and S.R.W., unpublished data; K. Weis, personal communication). IP₆ might serve as a stable cofactor for Gle1 activation of Dbp5; however, there is also the potential for regulation of inositide pools in response to extracellular stimuli. In yeast, Dbp5 is also functionally connected to Mex67, the yeast ortholog of TAP/NXF1. As *dbp5* mutants have increased levels of Mex67-associated RNA (Lund and Guthrie, 2005), localized activation of Dbp5 at the NPC cytoplasmic face is likely required to facilitate recycling of Mex67 or other mRNA bound proteins into the nucleus for subsequent rounds of mRNA export.

In Figure 3B, the striking parallel control mechanisms that spatially dictate transport factor activities for the Kap and mRNA export pathways are shown. At the NPC cytoplasmic face, the Kap pathway requires Ran GAP for stimulating Ran GTPase activity (reviewed in Fried and Kutay, 2003), and the mRNA export pathway requires Gle1 and IP₆ cofactors for stimulating Dbp5 ATPase activity. Thus, an NTPase activating protein is critical for both. In addition, juxtaposition of the respective proteins is mediated by specific Nup binding sites and enhanced by potentially regulatory factors (SUMO modification and IP₆, respectively).

Gene Gating: Functional Connectivity through the NPC

In addition to providing docking sites for enzymatic activities associated with transport, recent evidence now illustrates that Nups may regulate gene expression through functional connectivity in a process called “gene gating.” Gene gating was originally proposed in 1985 by Blobel (Blobel, 1985) and is based on the observation that NPCs do not distribute randomly around the nuclear envelope. This arrangement was predicted to mirror the distribution of transcriptionally active chromatin within the nucleus,

aiding in reformation of the nuclear envelope following mitosis as well as providing proper NPC distribution. Additionally, it would create a targeted pathway for export of mRNA transcripts to specific NPCs during interphase. It is now clear from multiple reports that certain Nups play an active role in both mitosis and NPC assembly (reviewed in Hetzer et al., 2005). However, only in the last few years have Nups been proposed to function in tethering transcriptionally active genes to the NPC.

The strongest support for the gene gating proposal comes from two reports by Silver and coworkers. Using ChIP assays and DNA microarray analysis, this report demonstrated that certain Nups associated preferentially with transcriptionally active genes in budding yeast (Casolari et al., 2004). Strikingly, genes under the control of the *GAL* promoter relocated to the nuclear periphery upon induction of transcription at these gene loci. This relocation was also observed for genes induced by addition of the α mating factor (Casolari et al., 2005). Both of these results correlated directly with alterations in gene expression.

Prior to these studies, however, several reports had linked components of the NPC to types of chromatin silencing such as boundary activity (the ability to prevent the spread of heterochromatin) (Ishii et al., 2002). How then can components of the NPC be linked to such opposing roles in gene expression? By examining the role of Nup2 in yeast, a protein previously shown to exhibit boundary activity when artificially tethered to the chromosome (Ishii et al., 2002), this precise question was recently addressed. Utilizing yeast DNA microarrays containing intergenic regions, in contrast to the ORF-specific microarrays used previously (Casolari et al., 2004, 2005), Aitchison and coworkers have found that Nup2 associates with chromatin at regions between heterochromatin and euchromatin (Dilworth et al., 2005). These investigators conclude that the chromatin association is mediated by formation of a tetrameric complex including Nup2, Nup60 (a nuclear basket component), Prp20 (yeast RanGEF), and a histone variant, Htz1 (Dilworth et al., 2005 and references therein). Htz1 has been proposed to function in boundary activity (Meneghini et al., 2003).

Using an innovative method of chromatin mapping, Laemmli and coworkers have pinpointed the interaction of Nup2 with the promoters of activated genes. This association is dependent on upstream transcriptional activating sequences and the TATA box (Schmid et al., 2006). The Laemmli and Aitchison studies now provide the first connection between Nups and factors that control transcription. Moreover, Nup interactions with promoters may be an important mechanism for control of gene expression through a modified model of the gene gating theory. In this model, the NPC would function as a switchboard for gene expression regulation by providing a sorting site for activities such as transcriptional activation and mRNA quality control (Figure 2). This functional connectivity would enable entire gene expression net-

works as well as regulatory elements to be linked from gene to cytoplasm through the NPC.

Given that all of the current supporting evidence for gene gating has been found in budding yeast, it leads one to question the evolutionary conservation of this phenomenon. In fact, cellular imaging of mammalian cells has revealed that mRNPs move within the nucleus primarily by diffusion (Shav-Tal et al., 2004). This finding would suggest a lack of physical gating of gene transcripts to the NPC. However, a recent report now illustrates that the NPC components are essential factors required for dosage compensation in *Drosophila*, a phenomenon characterized by hypertranscription of the hemizygous male X chromosome (Mendjan et al., 2006). This work is consistent with gene gating in higher eukaryotes; however, more experiments are necessary to determine if gene gating is indeed a ubiquitous, evolutionarily conserved mechanism for gene expression.

Evolution of Today's NPC

The fact that a single complex (and its components) can function in multiple different activities both directly and indirectly linked to its primary cellular role makes unraveling the origin of the modern day NPC challenging. As has been proposed for the ribosome (Moore, 1993), the NPC is likely the result of fusion of multiple distinct activities followed by adaptation and refinement. The first of these activities would include both diffusive permeability and selective transport. The ability to tether transport-specific effectors and directly control transport events would have been later additions that were preserved during evolution as activities that influence transport efficiency.

As a continuous nuclear envelope dividing the chromatin from the cytosol clearly cannot function without a transport portal, it is likely that the first rudimentary pore and any associated Nups would have originated in the first eukaryote upon creation of the first nucleus. However, recent extensive phylogenetic analyses have illustrated that several components of the soluble transport machinery, including Ran and Kaps, may be of bacterial origin (Mans et al., 2004). Notably absent in bacteria are ancestral homologs of Nups, although characteristic WD40 and TPR repeats are present. These findings led Koonin and coworkers to propose that the NPC was pieced together by contributions of domains from bacterial proteins (Mans et al., 2004). As this report notes, the absence of NPC components in bacteria is not supportive of an endosymbiotic origin for the nucleus. Therefore, the critical unanswered question is: what was the evolutionary trigger that sparked formation of double-membrane compartments characteristic of eukaryotes? In contrast, identification of a shared domain structure, namely the β propeller- α solenoid arrangement, between both components of both the NPC and COPII Golgi-mediated transport vesicles suggests a common evolutionary origin (Devos et al., 2004, 2006). In fact, this finding implicates a "proto-coatomer" ancestor of

clathrin-coated vesicles whose evolutionary divergence resulted in components of both vesicular transport and the NPC. However, the profound lack of sequence conservation is not supportive of a divergent evolutionary model for two relatively recent adaptations. Instead, we support a convergent evolutionary model whereby bacterial motifs such as the WD40 β propeller fold were used in two independent membrane bound structures.

Interestingly, the FG motifs characteristic of all eukaryotic NPCs, are not observed in bacteria and are likely a eukaryotic invention present in the last common eukaryotic ancestor (Mans et al., 2004). This suggests that early nuclear diffusive permeability barriers did not contain FG motifs. In fact, for the modern-day NPC, the non-FG Nups play key roles in forming the foundations of the permeability barrier. Whereas deletion of FG domains themselves has not been shown to impact diffusive permeability (Strawn et al., 2004), the absence of different non-FG Nups (yeast Nup170 or Nup188, and *C. elegans* Nup205 or Nup93) results in "leaky pores" (Shulga et al., 2000; Galy et al., 2003). Instead, FG motifs may have coevolved along with gene duplication and diversification of the Kap-based transport system, yielding the 20 different importin β paralogs and 10 or more FG Nups found in human cells today. This proposed scenario is consistent with the fact that bulk FG mass is not required for transport but specific FG Nups are necessary for distinct transport pathways (Strawn et al., 2004). However, given the lack of a true ancestral archetype, the evolutionary origin of the NPC is likely to remain speculative.

Future Challenges

Given that the NPC is a dynamic structure that can adapt to both transport status and cellular cues, a future challenge to the field will be the establishment of a testable model for nuclear transport. This model must incorporate all of the following: not all NPCs are identical, the NPC actively rearranges both during transport and during the cell cycle, there are multiple different transport pathways through the NPC, and transport-associated activities can be coupled to the NPC. Clearly, this is a daunting challenge and requires studies in multiple experimental systems including both in vitro and whole animal models. A more immediate challenge, however, is understanding the coordination between transport and other cellular functions of Nups. As components of the NPC are clearly involved in different stages of mitosis, their precise roles in cell cycle, as well as their potential functional connections to transport, remain to be determined. In fact, a complete functional catalog of Nups involved in mitosis may reveal intricate connectivities between reformation of the NPC as well as reestablishment of transport following mitosis, as originally suggested in the gene gating hypothesis (Blobel, 1985). Finally, with the "static NPC" model vanquished, investigations of Nups as regulatory entities may reveal elusive disease mechanisms.

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