Minireview

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The Nuclear Pore Complex as a Transport Machine*

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Nucleocytoplasmic Transport and the Nuclear Pore Complex

Unlike their prokaryotic counterparts, eukaryotic cells separate the nuclear synthesis of DNA and RNA from cytoplasmic protein synthesis with a barrier termed the nuclear envelope (NE).[1] The NE is perforated by large proteinaceous assemblies, called nuclear pore complexes (NPCs), which act as the sole gatekeepers controlling the exchange of material between the two locales (reviewed in Ref. 1). NPCs are freely permeable to small molecules (such as water and ions), but they restrict the movement of larger molecules (such as proteins and RNAs) across the NE. To overcome this barrier, macromolecules carry specific signals that allow them to access the nucleocytoplasmic transport machinery of the cell. In this way the cell ensures that only selected macromolecules can travel between the nucleus and cytoplasm (reviewed in Ref. 2).

Operationally, NPCs are composed of proteins called nucleoporins (or Nups) forming the stationary phase for nucleocytoplasmic exchange, whereas the mobile phase consists of soluble transport factors and their cargoes. As nucleocytoplasmic transport is driven by a series of specific interactions between components of both phases, it is frequently difficult to determine which proteins are permanent constituents of the NPC. Nevertheless, to understand how transport occurs, we must characterize the players in both phases and understand how their interplay leads to the coordinated vectorial exchange of macromolecules across the NE. In this review we focus on recent results that shed light on how some of these proteins interact to contribute to the elaborate NPC architecture and its function as a transport machine.

Architecture of the NPC

NPCs from different organisms share a common fundamental architecture (3). These similarities likely provide clues as to the key features common to a functioning transport machine. The NPC is a large octagonally symmetric cylindrical structure. In yeast it estimated to be ~50 MDa, whereas in metazoans it is over twice this mass (3, 4). Given that a ribosome at 4 MDa contains ~80 proteins, it might be expected that the NPC would contain hundreds of different nucleoporins. However, it has recently been shown that the yeast NPC contains only ~30 different proteins and the vertebrate NPC contains perhaps a few more (5). This raises the question of how such a large complex can be constructed from so few component parts. The answer appears to lie in the symmetry of the structure (Figs. 1–3). The NPC is comprised of a cylindrical core from which numerous peripheral filaments project toward the nucleus and cytoplasm (reviewed in Ref. 6) (Figs. 1 and 2). The remarkable symmetry of the NPC is most apparent in the central core. Not only is it composed of eight identical spokes, but each spoke is also seemingly mirror symmetrical both in a plane parallel to the NE and in a perpendicular plane running through the cylindrical axis. As predicted from this symmetry, all nucleoporins examined thus far are present in multiple copies (apparently 1, 2, or 4 copies per spoke and hence 8, 16, or 32 copies per NPC), and most are localized to both the nuclear and cytoplasmic sides of the NE (5–7) (Fig. 3). By combining this symmetry with the relatively large size of most known nucleoporins (generally between 50 and 360 kDa), it becomes clear how the massive NPC can actually be constructed from a comparatively small number of proteins. Furthermore, the large size of nucleoporins potentially allows them to span between more than one domain of the NPC. However, for simplicity, we will begin by considering each major morphological NPC domain in turn and examine how they may combine to form the complete functional machine.

The Pore Membrane Domain and Formation of the Nuclear Pore

The nuclear envelope is composed of three biochemically distinct domains. The outer NE membrane is continuous with the endoplasmic reticulum and the inner membrane lies within the nucleus. Nuclear pores are created by a fusion of these two membranes, thus defining the third membrane domain, the pore membrane. The resulting channel connects the nucleoplasm with the cytoplasm, and integral membrane proteins localized to this domain are termed Poms (pore membrane proteins). Although surprisingly little is known about the function of each Pom, they likely play a central role in NPC assembly by initiating the formation of the pore membrane domain, stabilizing it, and serving as a membrane anchor site for the growing NPC. Remarkably, little homology has been found so far between Poms from different organisms, but as the mechanism of pore formation is probably conserved, it seems likely that such homologues exist and have yet to be identified.

NPCs assemble continuously throughout interphase (8, 9); thus, the formation of the pore membrane domain must be fast and coincide with the insertion of the NPC, so that neither the nucleoplasm nor the ER lumen leaks during this process. Early assembly intermediates clearly have a pore membrane domain but apparently very little else and are presumably stabilized by integral pore membrane proteins such as Pom121p, which is recruited early in the reassembly process (10, 11). Gp210, a pore membrane protein known to be a major constituent of the lumenal ring, is apparently recruited later in NPC assembly (11). Gp210 is also hyperphosphorylated at the early stages of mitosis, and this modification may be important to initiate the mitotic disassembly of the NPC and nuclear envelope (12). Although Gp210 is a major protein in metazoan NPCs, the lack of an orthologue in yeast further suggests a possible role for Gp210 in NPC disassembly, as there is no NE disassembly step during yeast mitosis.

The Central Core: The Spoke-Ring Complex and Central Transporter

The core of the NPC is considered to be the compact, highly symmetrical framework that underlies and stabilizes the central structure of the NPC. As might be expected, all the yeast nucleoporins that seem to fit within this category are relatively abundant components, localized to both faces of the NPC (5, 7). Surprisingly, only one-third of all the core nucleoporins is essential in yeast. This is likely a result of the symmetry and compact organization of the central core, such that proteins within this region make multiple contacts with each other and contribute to an interwoven framework that is stable to the loss of any individual component. This idea is supported by various genetic and biochemical data. One of

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[1] The abbreviations used are: NE, nuclear envelope; NPC, nuclear pore complex; Nup, nucleoporin; RNP, ribonucleoprotein; NLS, nuclear localization signal.

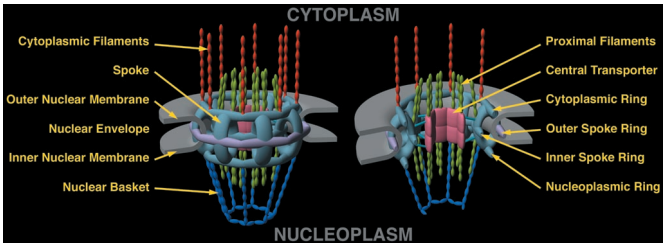

FIG. 1. **Structure of the nuclear pore complex.** Each NPC is a large proteinaceous assembly embedded in the pore membrane domain of the *nuclear envelope*, where the *inner* and *outer nuclear membranes* fuse. The NPC contains eight *spokes*, projecting radially from the wall of the pore membrane and surrounding a central tube called the *central transporter*. Each spoke is composed of numerous struts and attached to its neighbors by four coaxial rings: an *outer spoke-ring* in the lumen of the NE adjacent to the pore membrane, a *nucleoplasmic ring*, a *cytoplasmic ring*, and an *inner spoke-ring* surrounding the central transporter. A considerable portion of each spoke traverses the pore membrane and resides in the NE lumen. Together these structures comprise the central core. Peripheral elements project from this core toward the *nucleoplasm* and *cytoplasm*. These include: numerous *proximal filaments* on both faces of the cylindrical central core, whose presence (though not directly imaged) is inferred from the large number of symmetrically disposed filamentous nucleoporins; eight *cytoplasmic filaments*, attached at the cytoplasmic ring; and nuclear filaments originating at the nuclear ring and conjoining distally to form the *nuclear basket,* which connects with elements of the nucleoskeleton (not shown).

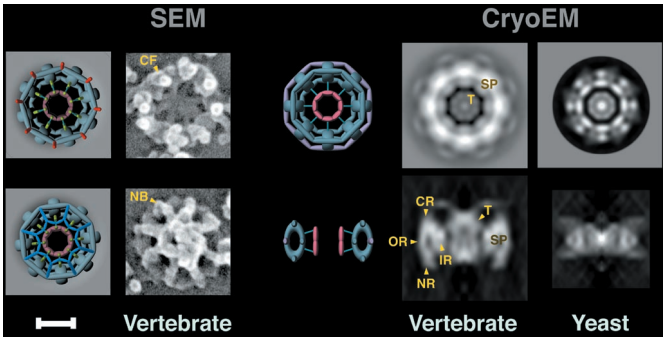

FIG. 2. **Visualization of NPC substructures.** Scanning electron microscopy (*left*) of a vertebrate (*Xenopus*) NPC viewed *en face* from the cytoplasm best reveals the cytoplasmic filaments (*CF*); an NPC viewed similarly from the nucleoplasm shows the nuclear basket (*NB*). The structures of the central core are revealed by three-dimensional protein density maps generated by cryoelectron microscopy and image processing (*CryoEM*, *right*) of both vertebrate and yeast NPCs. The positions of the spoke (*SP*) and central transporter (*T*) are indicated on both the *en face* projection map (*top row*) and longitudinal slice (*bottom row*) of the vertebrate NPC. The positions of the cytoplasmic ring (*CR*), nuclear ring (*NR*), outer spoke-ring (*OR*), and inner spoke-ring (*IR*) are indicated on a longitudinal slice. *Diagrams* at the *left* of the micrographs show the corresponding orientation of the NPC. Micrographs were kindly provided by Martin Goldberg and Terry Allen (*SEM*) and Chris Akey (*CryoEM*). *Bar*, 50 nm.

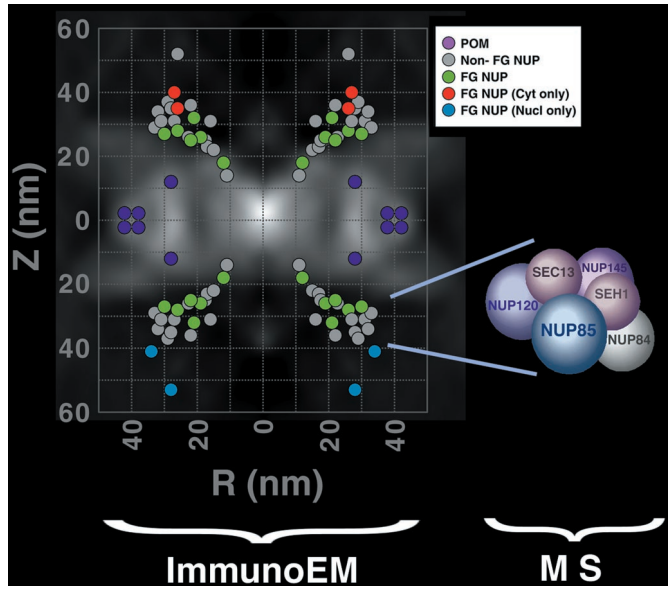

FIG. 3. **Increasing resolution maps of the NPC substructure.** Immunoelectron microscopy (*ImmunoEM*) has begun to map the position of the nucleoporins within the NPC, whereas mass spectrometry (*MS*) is one of the new techniques being used to map the direct interactions between individual nucleoporins (5, 14).

the best examples for this connectivity is the well defined six-member Nup84p subcomplex (Fig. 3) (13). Most proteins in this complex make interactions with several of their neighbors, creating a network of protein interactions stabilizing the overall structure (14). That only two members of the complex are essential in yeast may reflect this stability, such that the complex can suffer loss of components without catastrophic consequences. When examined by electron microscopy, the complex has a Y-shaped morphology and a mass of ~375 kDa. All the members are symmetrically disposed within each NPC and present in an estimated 16 copies (5, 13). Thus, this one subcomplex alone could potentially account for ~6 MDa of the 50-MDa yeast NPC! How this complex connects to the rest of the NPC still remains unclear, although it has been suggested that the arms of the "Y" structure interconnect to form one of the internal rings of the NPC (13).

A detailed comparison of the core structure in vertebrates and yeast points to the presence of additional structures in vertebrates, including a radial arm and more elaborate nuclear and cytoplasmic rings (Fig. 2) (3, 7, 15). However, the features of the central transporter and spoke-ring complex are conserved between the two, as they are in all eukaryotes studied. At the molecular level, for known core components, there is also remarkable conservation. For example, mammalian Nup155 can functionally replace its orthologue, the yeast core protein Nup170p (16). The yeast Nup84p subcomplex also appears conserved; sequence comparisons suggest that most members of this subcomplex have metazoan orthologues, and a similar vertebrate complex can be isolated containing at least some mammalian counterparts of the yeast complex (17).

How does the core contribute to NPC function? As all macromolecular transport across the NE occurs through the central transporter, supported within the core of the NPC, the core is obviously essential to transport. However, the core must also 1) maintain the structural integrity of the NPC as a barrier to diffusion while simultaneously being sufficiently flexible to withstand morphological changes in the nuclear envelope, 2) support the stepwise NPC assembly process, and 3) accommodate large transported cargo. Indeed, the spoke-ring complex and the central transporter have been observed in different morphological states by electron cryomicroscopy (15, 18). These different conformations suggest a sequential dilation of the central transporter, progressing from a resting state permeable to only smaller molecules to the triggering of a fully dilated state by the passage of the largest transport cargoes. Similar dramatic conformational changes within the central transporter and nuclear basket have been observed during the transport of large particles such as ribonucleoprotein particles (RNPs) (19, 20).

Interestingly, in yeast, removal of the core components Nup170p and Nup188p increases the nonselective macromolecular permeability of the NPC (21). These results are the first to define nucleoporins involved in controlling diffusion through the NPC and suggest that these proteins are either part of the transporter itself or anchor proteins that are.

Nucleocytoplasmic Transport and the Peripheral Nucleoporins

The framework of the core also correctly positions the peripheral nucleoporins. These nucleoporins are considered accessible to the mobile phase of transport and thus play a more direct role in interacting with carriers and their cargoes. Cargoes destined for the nucleus carry a nuclear localization signal (NLS), whereas substrates to be exported from the nucleus harbor nuclear export sequence (reviewed in Ref. 2). The signals are, in turn, recognized by a structurally related family of soluble transport receptor proteins collectively termed karyopherins (kaps; also known as importins, exportins, and transportins) (reviewed in Refs. 2 and 22). Transport cargoes, such as nuclear proteins, messenger RNPs, tRNA, ribosomal proteins, ribosomal subunits, and small nuclear RNPs, have distinct NLSs or nuclear export sequences that are recognized by their own particular cognate transport factors. This interaction is controlled by the small GTPase Ran (see below and Refs. 23 and 24). Electron microscopy studies suggest that the karyo-

pherin-NLS-cargo complex docks at multiple sites along the cytoplasmic filaments and through the NPC (25, 26). Thus, it is proposed that nuclear import is facilitated by a series of karyopherin docking and release steps, as the cargo-carrier complex moves along peripheral nucleoporins from the cytoplasmic filaments of the NPC through the central transporter, to the nucleoplasmic face, where the complex is released to the nuclear interior (27).

FG Nucleoporins Provide an Abundance of Transport Factor Binding Sites at the NPC—Of course, to fully understand how the NPC might directly contribute to transport, it is necessary to first characterize its components. The NPC is crammed with nucleoporins characterized by the presence of the FG dipeptide (Phe-Gly) repeat motifs. These repeats are present in nearly half the nucleoporins and often take the form of GLFG or F*X*FG repeats, separated by polar sequences of varying lengths. These so-called FG nucleoporins appear to be built upon the core structure and are present throughout the NPC, extending from the tips of the cytoplasmic filaments through the central transporter to the distal ring of the nuclear basket (Fig. 3) (5, 7). As the FG nucleoporins are strategically positioned to be accessible to the mobile phase and interact directly with all of the karyopherins studied (as well as other cargo-carrying transport factors) (28), they are implicated directly in facilitating karyopherin/cargo movement across the NPC.

Analysis of the structure of an FG repeat region bound to a karyopherin indicates that multiple FG repeats likely interact with numerous conserved hydrophobic pockets running along the outside of the karyopherin via phenylalanines in the FG repeat. Overall, the FG repeat region adopts an extended conformation with little intrinsic secondary structure. Furthermore, FG nucleoporins have been shown to form filaments (30) and colocalize with the filamentous structures of the NPC (reviewed in Refs. 6 and 7). This is consistent with these proteins forming the majority of the filaments that emanate from the core and extend into the nucleoplasm and cytoplasm, although other possible conformational states cannot be excluded. As might be expected from their projection from the core of the NPC, in many cases FG nucleoporins are anchored to the core by one or the other of their ends (31–34).

Different Transport Factors, Different Docking Sites—Every transport factor studied can bind FG nucleoporins that have also been shown to bind other classes of transport factors (reviewed in Ref. 28). This fact and the observations that saturated or irreversible binding of some karyopherins to the NPC can be deleterious to other pathways suggest that pathways through the NPC overlap in specificity. However, considering the symmetry of the NPC and the abundance of FG nucleoporins there may be ~160 transport factor binding sites per NPC. Although this provides a multitude of possible binding sites for each transport factor molecule, karyopherins have strong preferences for a restricted subset of FG nucleoporins (reviewed in Ref. 28). This could allow different karyopherins to simultaneously occupy different sites within a single NPC, while limiting the competitive interference between different pathways and increasing the potential transport flux in both directions. Indeed, it has been shown that a single NPC is capable of both exporting and importing different transport substrates (35). The NPC could also use such docking specificity as a way to globally regulate gene expression by simply modifying a nucleoporin dedicated to a particular nuclear transport factor. One of the most intriguing examples of this is the interaction between Kap121p and Nup53p in yeast. Although *in vitro* binding studies and *in vivo* fluorescence resonance energy transfer measurements demonstrate that Kap121p interacts with several different FG nucleoporins while transiting the NPC, both studies suggest that Nup53p is a specific docking site for Kap121p (36, 37). Thus, although not absolute, it appears that Nup53p could confer control over the Kap121p-mediated import pathway. Interestingly, Nup53p is phosphorylated at mitosis, and there is a concomitant decrease in the binding of the karyopherin Kap121p to the NPC although it remains to be determined if this results in a specific cell cycle-dependent change in nuclear import (36).

The Strategic Positioning of the Docking Sites: Efficient and Directional Transport—From studies mapping the relative position of all the nucleoporins in yeast, it is striking that many FG nucleoporins are symmetrically disposed closely surrounding the central transporter, whereas FG nucleoporins localized exclusively to either the nucleoplasmic or cytoplasmic sides are placed further away from the core. This observation suggests that the directionality of transport factors through the NPC is conferred by the FG nucleoporins at the extremities of the NPC. It also likely that there are more subtle arrangements of docking sites within the symmetrical regions of the NPC in which precise order and distribution helps correctly direct transport factors as they transit the pore. Interestingly, like the core nucleoporins, complexes formed by the peripheral nucleoporins are also well conserved. Thus both the yeast Nsp1p nucleoporin subcomplex and the analogous vertebrate p62-p58-p54 complex are found on both sides of the NPC surrounding the central transporter (5, 38–40). This together with the fact that there is only minimum amino acid sequence conservation in the repeat motifs between presumed orthologues from different species (41) suggests that the conservation lies in the functionality of the conserved binding sites themselves and in their similar strategic positions within the NPCs of different organisms.

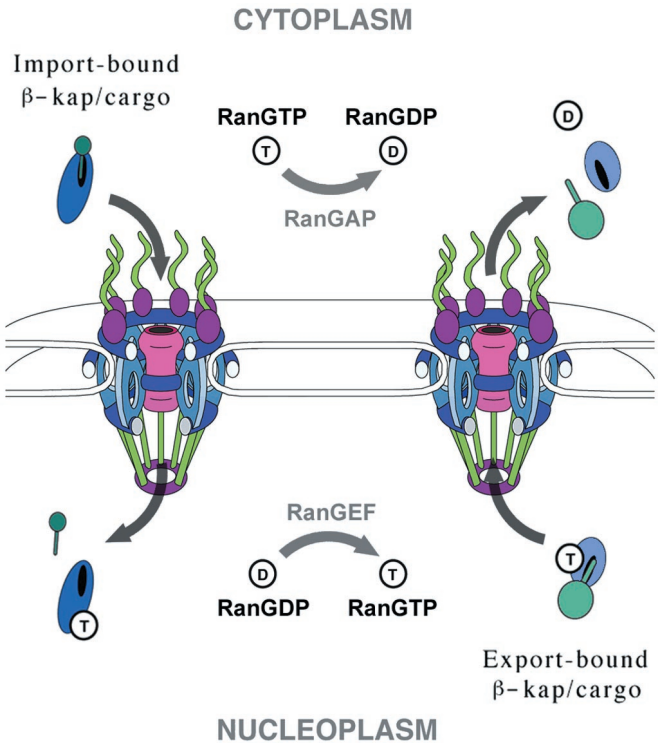

FIG. 4. **The Ran cycle.** Ran cycles between its GTP- and GDP-bound form dependent on its subcellular localization. The different forms of Ran confer directionality to transport by dictating where karyopherins bind and release their cargoes. See "The Energetics of Transport" for details. *D*, Ran-GDP; *T*, Ran-GTP.

The Energetics of Transport—In addition to nucleoporins, sustained karyopherin-mediated nucleocytoplasmic exchange requires energy. The only known source of this energy is the small GTPase Ran (reviewed in Refs. 23 and 24). However, as the translocation process itself is not linked to GTP hydrolysis, it is likely that the energy comes from a potential energy gradient across the NPC established by the maintenance of distinct pools of Ran: GTP-Ran in the nucleus and GDP-Ran in the cytoplasm (Fig. 4). This asymmetric distribution supports transport by triggering the assembly and disassembly of transport complexes in the correct compartments. Thus, importers release their cargoes when they interact with Ran-GTP in the nucleus, whereas exporters utilize Ran-GTP to bind their cargoes. Conversely, when the GTP on Ran is hydrolyzed (as is the case in the cytoplasm) importers can bind their cargoes, but exporters will release theirs.

Although Ran is soluble in both the nucleoplasm and cytoplasm, it is also directly tethered to the NPC through at least two different protein motifs within nucleoporins. The first domain is homologous to the cytoplasmic Ran-binding protein RanBP1. This domain binds both Ran-GTP and Ran-GDP and has been found in the cytoplasmic FG nucleoporin Nup358p (42). The second type of Ran binding domain, characterized by a zinc finger motif, binds Ran-

GDP and is present on both Nup358p (43) and the nucleoplasmically disposed nucleoporin Nup153p (44). Ran binding to these distal nuclear and cytoplasmic components of the NPC may ensure a high concentration of Ran in the vicinity of the nuclear pore, improving the efficiency of the transport termination steps. This role may involve promoting the exchange of Ran between transport factors and maintaining the Ran-GTP/Ran-GDP gradient across the NPC. In addition, Nup358 tethers Ran-GAP (which activates the GTPase activity of Ran) to the NPC (45, 46). Localizing karyopherin docking sites, Ran binding sites, and Ran-GAP to the same nucleoporin may provide a means of ensuring highly efficient loading and unloading of transport factors and their cargoes during transport. This tethering may be particularly important in the relatively large mammalian cells where soluble factors have the potential to diffuse great distances away from the pore. In this respect it is interesting that in the smaller yeast cells, the only Ran-binding protein known to associate with the NPC (Nup2p (47)) is dispensable, suggesting that the presence of such domains at the NPC may not be an absolute requirement for transport.

The export of messenger ribonucleoprotein complexes seems to require additional cofactors at the NPC with their cognate nucleoporin binding sites. Thus, the RNA helicase Dbp5p is associated with both yeast Nup159p and its vertebrate homologue Nup214; the RNP-binding protein Gle2p is similarly bound to the NPC (reviewed in Ref. 28). These proteins may aid in the quaternary structural changes in the RNP necessary to wind it through the narrow central transporter and, in the case of the ATP-driven helicase, provide additional energy for the translocation of the comparatively huge RNP particles across the NPC.

The Mechanism of Transport

Four major principles must be considered to develop a model for transport through the NPC. First, the NPC provides a barrier to the diffusion of macromolecules across the NE, but it must also be gated to permit the rapid passage of macromolecules bearing the appropriate signals. As there appears to be no NTP-driven mechanism to promote the dilation of the channel, the apparent opening and closing of the channel may not be actively gated by NPC components. The second important principle is that the narrow diameter of the channel and the Brownian motion of the flanking, closely spaced, filamentous nucleoporins likely make macromolecular diffusion across the NPC entropically unfavorable. Third, transport across the NPC is mediated by a multitude of cargo-carrying transport factors that interact with the large number of FG nucleoporins. One model proposes that the entropic exclusion of the NPC can be overcome, in the case of transport factor-cargo complexes, by the energy associated with their binding to the FG nucleoporins themselves. As most of these FG nucleoporins are equally distributed on both sides of the NE, transport factors could then readily exchange between nucleoporins on both sides of the NE. The NPC is therefore effectively a "virtual gate"; as proteins that can bind the NPC pass the diffusion barrier of the central channel much more freely than those that do not, gating selectivity is achieved without necessarily invoking a gate composed of any moving parts (in the conventional sense) (5). The fourth principle is that asymmetric, high affinity binding sites particularly at the extreme nuclear and cytoplasmic faces of the NPC likely contribute (together with the Ran GTP/GDP gradient) to determining the directionality of transport (5). Indeed several lines of evidence indicate that a high affinity terminal nucleoporin binding step contributes to the transport directionality of karyopherin-cargo complexes (48–50).

Summary and Perspectives

The work discussed here still represents only a promising beginning, and two major challenges remain. First, it remains necessary to determine the nature of the regulated interactions and connectivity of the mobile and stationary phase components and to test various models to understand in detail how these two phases interface to regulate macromolecular transport across the nuclear envelope. Second, we must establish what alterations and additions evolution has provided to build nucleocytoplasmic transport systems capable of responding to the various needs of cells found within the wide variety of eukaryotic organisms.

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REFERENCES

1. Wente, S. R. (2000) *Science* **288,** 1374–1377
2. Mattaj, I. W., and Englmeier, L. (1998) *Annu. Rev. Biochem.* **67,** 265–306
3. Yang, Q., Rout, M. P., and Akey, C. W. (1998) *Mol. Cell* **1,** 223–234
4. Reichelt, R., Holzenburg, A., Buhle, E. L., Jr., Jarnik, M., Engel, A., and Aebi, U. (1990) *J. Cell Biol.* **110,** 883–894
5. Rout, M. P., Aitchison, J. D., Suprapto, A., Hjertaas, K., Zhao, Y., and Chait, B. T. (2000) *J. Cell Biol.* **148,** 635–651
6. Allen, T. D., Cronshaw, J. M., Bagley, S., Kiseleva, E., and Goldberg, M. W. (2000) *J. Cell Sci.* **113,** 1651–1659
7. Stoffler, D., Fahrenkrog, B., and Aebi, U. (1999) *Curr. Opin. Cell Biol.* **11,** 391–401
8. Maul, G. G., Price, J. W., and Lieberman, M. W. (1971) *J. Cell Biol.* **51,** 405–418
9. Winey, M., Yarar, D., Giddings, T. H., Jr., and Mastronarde, D. N. (1997) *Mol. Biol. Cell* **8,** 2119–2132
10. Gant, T. M., Goldberg, M. W., and Allen, T. D. (1998) *Curr. Opin. Cell Biol.* **10,** 409–415
11. Bodoor, K., Shaikh, S., Salina, D., Raharjo, W. H., Bastos, R., Lohka, M., and Burke, B. (1999) *J. Cell Sci.* **112,** 2253–2264
12. Favreau, C., Worman, H. J., Wozniak, R. W., Frappier, T., and Courvalin, J. C. (1996) *Biochemistry* **35,** 8035–8044
13. Siniossoglou, S., Lutzmann, M., Santos-Rosa, H., Leonard, K., Mueller, S., Aebi, U., and Hurt, E. (2000) *J. Cell Biol.* **149,** 41–54
14. Rappsilber, J., Siniossoglou, S., Hurt, E. C., and Mann, M. (2000) *Anal. Chem.* **72,** 267–275
15. Akey, C. W., and Radermacher, M. (1993) *J. Cell Biol.* **122,** 1–19
16. Aitchison, J. D., Rout, M. P., Marelli, M., Blobel, G., and Wozniak, R. W. (1995) *J. Cell Biol.* **131,** 1133–1148
17. Fontoura, B. M., Blobel, G., and Matunis, M. J. (1999) *J. Cell Biol.* **144,** 1097–1112
18. Akey, C. W. (1990) *Biophys. J.* **58,** 341–355
19. Kiseleva, E., Goldberg, M. W., Daneholt, B., and Allen, T. D. (1996) *J. Mol. Biol.* **260,** 304–311
20. Kiseleva, E., Goldberg, M., Allen, T., and Akey, C. (1998) *J. Cell Sci.* **111,** 223–236
21. Shulga, N., Mosammaparast, N., Wozniak, R., and Goldfarb, D. S. (2000) *J. Cell Biol.* **149,** 1027–1038
22. Wozniak, R. W., Rout, M. P., and Aitchison, J. D. (1998) *Trends Cell Biol.* **8,** 184–188
23. Cole, C. N., and Hammell, C. M. (1998) *Curr. Biol.* **8,** R368–R372
24. Moore, M. S. (1998) *J. Biol. Chem.* **273,** 22857–22860
25. Akey, C. W., and Goldfarb, D. S. (1989) *J. Cell Biol.* **109,** 971–982
26. Richardson, W. D., Mills, A. D., Dilworth, S. M., Laskey, R. A., and Dingwall, C. (1988) *Cell* **52,** 655–664
27. Blobel, G. (1995) *Cold Spring Harbor Symp. Quant. Biol.* **60,** 1–10
28. Ryan, K. J., and Wente, S. R. (2000) *Curr. Opin. Cell Biol.* **12,** 361–371
29. Bayliss, R., Littlewood, T., and Stewart, M. (2000) *Cell* **102,** 99–108
30. Buss, F., Kent, H., Stewart, M., Bailer, S. M., and Hanover, J. A. (1994) *J. Cell Sci.* **107,** 631–638
31. Enarson, P., Enarson, M., Bastos, R., and Burke, B. (1998) *Chromosoma* **107,** 228–236
32. Del Priore, V., Heath, C., Snay, C., MacMillan, A., Gorsch, L., Dagher, S., and Cole, C. (1997) *J. Cell Sci.* **110,** 2987–2999
33. Grandi, P., Schlaich, N., Tekotte, H., and Hurt, E. C. (1995) *EMBO J.* **14,** 76–87
34. Schlaich, N. L., Haner, M., Lustig, A., Aebi, U., and Hurt, E. C. (1997) *Mol. Biol. Cell* **8,** 33–46
35. Dworetzky, S. I., Lanford, R. E., and Feldherr, C. M. (1988) *J. Cell Biol.* **107,** 1279–1287
36. Marelli, M., Aitchison, J. D., and Wozniak, R. W. (1998) *J. Cell Biol.* **143,** 1813–1830
37. Damelin, M., and Silver, P. A. (2000) *Mol. Cell* **5,** 133–140
38. Guan, T., Muller, S., Klier, G., Pante, N., Blevitt, J. M., Haner, M., Paschal, B., Aebi, U., and Gerace, L. (1995) *Mol. Biol. Cell* **6,** 1591–1603
39. Grote, M., Kubitscheck, U., Reichelt, R., and Peters, R. (1995) *J. Cell Sci.* **108,** 2963–2972
40. Fahrenkrog, B., Hurt, E. C., Aebi, U., and Pante, N. (1998) *J. Cell Biol.* **143,** 577–588
41. Fabre, E., and Hurt, E. (1997) *Annu. Rev. Genet.* **31,** 277–313
42. Wu, J., Matunis, M. J., Kraemer, D., Blobel, G., and Coutavas, E. (1995) *J. Biol. Chem.* **270,** 14209–14213
43. Yaseen, N. R., and Blobel, G. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 5516–5521
44. Nakielny, S., Shaikh, S., Burke, B., and Dreyfuss, G. (1999) *EMBO J.* **18,** 1982–1995
45. Mahajan, R., Delphin, C., Guan, T., Gerace, L., and Melchior, F. (1997) *Cell* **88,** 97–107
46. Matunis, M. J., Wu, J., and Blobel, G. (1998) *J. Cell Biol.* **140,** 499–509
47. Dingwall, C., Kandels-Lewis, S., and Seraphin, B. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 7525–7529
48. Gorlich, D., Pante, N., Kutay, U., Aebi, U., and Bischoff, F. R. (1996) *EMBO J.* **15,** 5584–5594
49. Floer, M., and Blobel, G. (1999) *J. Biol. Chem.* **274,** 16279–16286
50. Kehlenbach, R. H., Dickmanns, A., Kehlenbach, A., Guan, T., and Gerace, L. (1999) *J. Cell Biol.* **145,** 645–657