New Ways to Skin a Kap: Mechanisms for Controlling Nuclear Transport

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Abstract

Transport between the nucleus and the cytoplasm occurs through large macromolecular assemblies called nuclear pore complexes (NPCs). The NPC is traditionally viewed as a passive structure whose primary role is to provide an interface for the soluble transport machinery, the karyopherins and their cargos, to move molecules between these compartments. Recent work has challenged this view of the NPC and provides support for a dynamic structure that can modify its architecture to actively regulate nuclear transport.

Introduction

In eukaryotic cells, the contents of the nucleus are physically separated from the cytoplasm by an impermeable double membrane called the nuclear envelope (NE). This separation of the contents of the nucleus, including the cell's chromatin and transcriptional machinery, from the cytoplasm, containing the cell's translational machinery, demands that cells regulate a vast array of macromolecular traffic between these compartments. All this traffic occurs through nuclear pore complexes (NPCs), which extend across the NE and act as gatekeepers for all transport. The NPCs function in concert with a family of soluble factors, termed karyopherins or kaps (also termed importins or exportins) that recognize cargos by binding a nuclear localization signal (NLS) or a nuclear export signal (NES) in either the cytoplasm or nucleus, respectively, and docks them to the NPC for subsequent translocation (Macara, 2001; Fried and Kutay, 2003). Individual karyopherins have the ability to bind specific classes of cargos, thus providing the cell with a means to independently regulate the transport of different classes of molecules including most proteins and ribonucleoprotein particles between the cytoplasm and the nucleoplasm (reviewed in Fried and Kutay, 2003). Understanding the mechanisms that underlie these pathways is paramount to understanding normal cellular function, including how cells regulate gene expression, progress through the cell cycle, transduce both intra- and extra-cellular signals, and how viruses gain access to the nucleus for productive infection.

Current models of nuclear transport come from studying both the NPC itself and kaps that recognize molecules to be transported through the NPC. The NPC is a huge, extraordinarily complex octagonally symmetric structure. In vertebrates, its mass has been estimated at ~60 million Daltons (Cronshaw et al., 2002). By comparison, the mass of the yeast NPC is estimated at ~44 million Daltons (Rout et al., 2000). Morphologically, this difference appears to be due to the more complex cytoplasmic and nucleoplasmic rings in vertebrate NPCs. However, the majority of the structure is extremely well conserved (Yang et al., 1998). This conservation is such that many proteins involved in nuclear transport are conserved and some NPC proteins (termed nucleoporins or nups) can function across phyla from yeast to mammals (Aitchison et al., 1995b)

In recent years, biochemical and genetic approaches have led to the identification of most, if not all, nups (reviewed in Suntharalingam and Wente, 2003). These studies have culminated with mass spectrometry (MS) analyses performed on enriched fractions of yeast and rat NPCs that suggest NPCs are composed of ~30 nups (Rout et al., 2000; Cronshaw et al., 2002) many of which are conserved between species. Nups can be divided into three subgroups. One group is composed of integral membrane proteins that are believed to play a role in NPC assembly and anchoring the NPC to the membrane. The two remaining groups are divided based on the presence or absence of repeated peptide motifs of the type GLFG, FXFG, PSFG or FG. The 'FG-nups', consisting of ~12 members, play a direct role in transport and several members have been shown to physically interact with karyopherins (reviewed in Ryan and Wente, 2000). The FG-nups have been detected throughout the NPC thus creating a series of kap binding sites along the ~200 nm pathway that extends from the tips of the cytoplasmic filaments to intranuclear fibres that form a structure called the nuclear basket (Suntharalingam and Wente, 2003). It has been estimated that there are ~160 FG-nucleoporins per NPC, many of which contain multiple FG-repeats (Rout et al., 2000, Strawn et al., 2004). Thus, the NPC is literally lined with kap binding sites.

Functions for non-repeat nucleoporins are less clear. These proteins, which are the most evolutionarily conserved of the nups, are considered to be integral to NPC assembly and are thought to provide the scaffold on which the FG-nups are organized. Consistent with this idea, a vertebrate NPC subcomplex consisting largely of non-repeat nups, including Nup107 and Nup160, has been shown to play a central role in the initial steps of NPC formation (Walther et al., 2003; Harel et al., 2003). The yeast counterpart of this complex has also been proposed to function in NPC assembly (Aitchison et al., 1995a; Doye and Hurt, 1997). Similarly, Nic96p and its vertebrate orthologue Nup93, are required for proper NPC formation and distribution along the surface of the NE (Zabel et al., 1996; Grandi et al., 1997; Galy et al., 2003). Nup170p is another non-repeat nup that appears to be a central player in organizing the FG-nups. In its absence there are changes in the localization and stoichiometry of several FG-nups including Nup1p, Nup53p and Nup2p (Kenna et al., 1996; Lusk et al., 2002). In fact, there appears to be a significant destabilization of NPC's lacking Nup170p, as many FG and non-FG nups alike are dissociated from nup170D NPCs in the presence of aliphatic alcohols or energy poisons (Shulga and Goldfarb, 2003). These effects also seem to manifest themselves by increasing the size of the diffusion channel through the NPC, an effect mimicked in nup188D strains (Shulga et al., 2000). Importantly, there are physical and genetic interactions between the non-FG and FG-nups, reinforcing the idea that while the non-repeat nups may not directly interact with kaps, they are nonetheless vital for nuclear transport.

The soluble transport machinery

The NPC controls constitutive transport by two basic mechanisms: (1) it acts as a passive diffusion barrier to molecules <9 nm in diameter and (2), through a more widely studied pathway, it mediates macromolecular traffic using kaps and other soluble factors including the small GTPase Ran (for reviews see Macara, 2001; Weis, 2003; Fried and Kutay, 2003). The advances in our understanding of macromolecular transport are the result of biochemical and genetic studies in metazoan cells and yeast. While certain nuances exist, the fundamental processes are conserved throughout all eukaryotes.

A broad spectrum of macromolecules cross the NE, including mRNAs, tRNAs, ribosomal proteins, ribosomal subunits, snRNPs and many soluble proteins. These various classes of molecules contain different NLSs or NESs. In most cases, they are recognized by different members of a family of structurally related kaps collectively referred to as ß-karyopherins (or ß-kaps), of which there are 14 in yeast that function primarily as either importers or exporters (Wozniak et al., 1998; Strom and Weis, 2001; Fried and Kutay, 2003). While the overall structure of the ß-kaps is believed to be

similar, their sequence similarity is low, except for a region near the N-terminus that contains a binding site for the GTPase Ran. The differences in their sequences likely reflect their ability to recognize different cargos. However, it is important to note that an individual signal can be recognized by more than one ß-kap suggesting some level of overlap between their functions. Examples are the ability of the ß-kaps Kap121p and Kap123p to both import the ribosomal protein rpl25 (Rout et al., 1997), and the need for Kap114p, Kap121p and Kap123p to import histones (Mosammaparast et al., 2001). Such examples may explain the observation in yeast, that deletions of some ß-kap genes are lethal while others are not.

In addition to the ß-kaps, nucleo-cytoplasmic exchange requires the activity of the GTPase Ran (Macara, 2001; Weis, 2003; Fried and Kutay, 2003). Ran is the only known energy source required for maintaining the transport cycle and the energy for transport likely comes from a potential energy gradient across the NPC established by the maintenance of distinct pools of Ran. In the nucleus, Ran is maintained in its GTP-bound state by the nuclear-restricted GTP exchange factor, Ran-GEF. In contrast, the Ran GTPase activating protein (Ran-GAP) is primarily cytoplasmic, ensuring that this pool of Ran is in its GDP-bound form. This distribution contributes to the directionality of transport by triggering the assembly and disassembly of transport complexes in the correct compartments. That is, the formation of import complexes between ß-kaps and their cargo is stable in the presence of cytoplasmic Ran-GDP. However, once the ß-kap/cargo complexes traverse the NPC and enter the nucleoplasm, Ran-GTP binds to the ß-kaps and displaces their cargo, terminating import. On the other hand, the formation of export complexes is stabilized in the nucleus by Ran-GTP and as these complexes reach the cytoplasm, the GTP is hydrolyzed and the complex disassembles. Moreover, the Ran-GTP gradient provides energy for recycling kaps back to the cytoplasm and continued rounds of transport.

Transport models

While the individual components of nuclear transport are well defined, the way in which they functionally interact to drive nuclear transport is not well understood. This is particularly true with respect to the role that kap-nup interactions play in moving the kap-cargo complex through the central channel of the NPC. In general, the binding of FG-nups to kaps is believed to facilitate the movement of the kap-cargo complex through the NPC. This idea has been incorporated into several nuclear transport models. One current model argues that, due to the hydrophobic nature of the FG-repeats and their abundance within the pore, FG-nups form a hydrophobic meshwork or "selective phase" that is impermeable to most molecules. Kaps together with their cargos, however, are able to partition into this matrix to facilitate their movement through the NPC (Ribbeck and Gorlich, 2001; Ribbeck and Gorlich, 2002). Another model envisions that the vast majority of molecules are excluded from the NPC channel due to their inherent entropy. The FG-nups are considered to be flexible disorganized proteins that occlude the central channel, and actively brush away molecules that cannot interact with them. In this fashion they further increase the entropic barrier of the NPC. By selectively interacting with kaps, the tentacle-like FG-nups also act as a "virtual gate" that allows the concentration of kap/cargo complexes within the NPC, lowering their entropy and allowing them to move through the pore (Rout et al., 2000; Rout et al., 2003).

In order to achieve rates of import sufficient to accommodate the huge flow of molecules across the NE, both of these models rely on the idea that the kap/nup interaction is weak (Ribbeck and Gorlich, 2001). Differences in affinity between kaps and FG-nups have been measured, however, and there is a tendency for the affinity of kaps for certain FG-nups to increase from the cytoplasmic filaments to the nuclear basket (Ben-Efraim and Gerace, 2001; Pyhtila and Rexach, 2003). These observations have been incorporated into an 'affinity gradient' model of nuclear transport, whereby kaps are pulled through the pore by an ever increasing affinity for nups along their route.

While these models provide a basic framework for

understanding transport, they represent broad strokes in what is likely a finely detailed landscape. For example, the models discussed above do not adequately incorporate a growing body of evidence that suggests that kaps do not all travel similar routes through the NPC. There are clearly specific binding sites, some of which are of high affinity, that have been proposed to play roles in regulating specific transport pathways. Furthermore, these models place tremendous emphasis on the FG-repeats as the main effectors of nuclear transport, which may, in fact, be an overstatement. As a case in point, a recent study evaluates the importance of individual FG-repeats in nuclear transport by systematically deleting FG regions of nups (Strawn et al., 2004). Amazingly, half of the total mass of FG's could be simultaneously deleted without dramatically affecting cell viability. Furthermore, while transport defects were observed, they were linked to specific transport pathways and did not represent a general inhibition of import and export processes. These data are therefore consistent with a model of nuclear transport that is more elaborate than those described above, supporting the idea that there are distinct transport routes through the NPC that are followed by specific karyopherins. They also underscore the involvement of non-FG binding sites in key transport roles (see below).

Regulation of nuclear transport

As a blueprint has emerged for the basic mechanisms of nuclear transport, so has the idea that specific transport pathways can be regulated to orchestrate changes in nuclear physiology including gene transcription, DNA replication, and chromosome segregation. The mechanisms governing this regulation can be broadly grouped into two categories: mechanisms that directly affect kap binding to cargo and those that encompass regulation of the kap-nup interaction. In this review, we will only highlight some of the mechanisms governing the kap-cargo interaction as they have been extensively reviewed elsewhere (Kaffman and O'Shea, 1999) and will focus on the regulation of transport by the NPC.

Regulating the kap-cargo interaction

Certainly the most well studied form of transport regulation relies on the modification or masking of an NLS or NES from its cognate kap, to prevent its import or export. Modifications such as phosphorylation and acetylation have been shown to either enhance or inhibit the binding of cargos to karyopherins (Kaffman and O'Shea, 1999; Madison et al., 2002). There is also recent evidence that implicates methylation as a key regulator of the import of certain NLSs (Smith et al., 2004). As an example, the distribution of the yeast transcription factor Pho4p is determined by binding to either the import ß-kap Kap121p or the export ß-kap Kap142p/Msn5p (Kaffman et al., 1998a; Kaffman et al., 1998b). Under phosphate-rich conditions Pho4p is phosphorylated, which results in the simultaneous inhibition of its interaction with Kap121p (import inhibition) and stimulation of its interaction with Kap142p/Msn5p (increased export), the end result being the exclusion of Pho4p from the nucleoplasm. Under conditions of phosphate-starvation, Pho4p becomes dephosphorylated which allows it to interact with Kap121p and breaks its interaction with Kap142p/Msn5p, driving the accumulation of Pho4p in the nucleus and subsequent transcriptional activation (Fig. 1). A reciprocal effect is observed with cyclin B1, which accumulates in the nucleus at the beginning of mitosis due to a block in export caused by its phosphorylation (Yang et al., 1998).

Several examples also exist where protein-protein associations between members of a complex can be altered to expose or hide a transport signal from a kap. p53, a well studied transcription factor linked to tumor suppression, dynamically shuttles between the nucleus and cytoplasm. When cells are stressed, p53 is tetramerized which enhances its ability to bind DNA and activate transcription. Interestingly, the tetramerization domain overlaps a leucine-rich NES that is recognized by the export kap Crm1p. Tetramerization of p53 thus hides the NES from Crm1p and effectively traps p53 in the nucleus, allowing it to continually promote transcription (Stommel et al., 1999)(Fig. 2).

Regulation of transport by the NPC

In addition to mechanisms that modulate interactions between kaps and their cargos, there is accumulated evidence supporting a role for the NPC in changing levels of nuclear transport. One can envision mechanisms that both globally alter the permeability of the NPC as well as those that affect the transport of specific karyopherins and their cargos. This concept requires one to reconsider the role of the NPC in regulating transport from a more traditional view of the NPC, as a passive constitutively active channel, to one that is more dynamic, capable of altering transport through changes in its structure. For example, permeability changes driven by molecular rearrangements of the NPC could occur in response to changes in cellular physiology. In support of this idea, evidence in BALB/c 3T3 cells suggests that the size of the NPC channel can be significantly altered. When comparing nuclear transport between proliferating and quiescent cells, Feldherr and Akin (1993) observed that the size of the NPC translocation channel was larger in cells that were actively growing. They also detected changes in NPC permeability throughout the cell cycle, reinforcing the idea that the NPC can gear its transport capability to elicit cellular functions (Feldherr and Akin, 1994). The molecular mechanisms causing these changes, however, are not yet known.

Along similar lines, it is hypothesized that cells may regulate transport by altering the complement of nucleoporins in NPCs. This has been observed at both the level of individual NPCs as well as in the tissue-specific expression of certain nup genes. Mlp1p, for example, is absent from NPCs surrounding the nucleolus in yeast. The functionality of this unique distribution is not yet clear, but is proposed to link transcription with mRNA export by promoting the nuclear retention of unspliced mRNAs in regions associated with chromatin (Galy et al., 2004). Importantly, these data raise the exciting possibility that cells can regulate the function of individual pores and, therefore, their ability to transport specific cargos. This idea may also be relevant to the unique tissue-expression

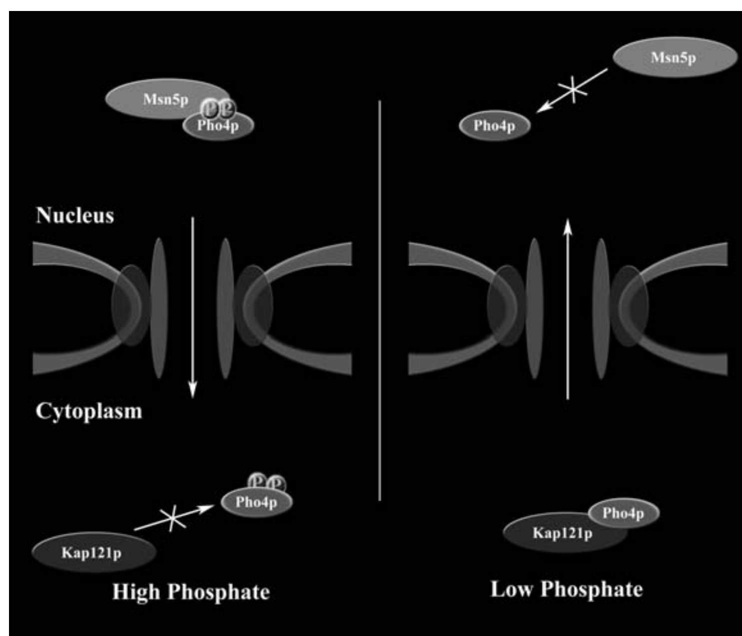

Figure 1. The regulation of Pho4p transport. Under growth conditions in which the concentration of phosphate ions is high, Pho4p is phosphorylated (P). The phosphorylation of Pho4p inhibits its interaction with the import karyopherin Kap121p, while enhancing its interaction with the export karyopherin Msn5p, resulting in a steady state accumulation of Pho4p in the cytoplasm. The reciprocal occurs when the concentration of phosphate ions are lowered, which results in the dephosphorylation of Pho4p and its accumulation in the nucleus.

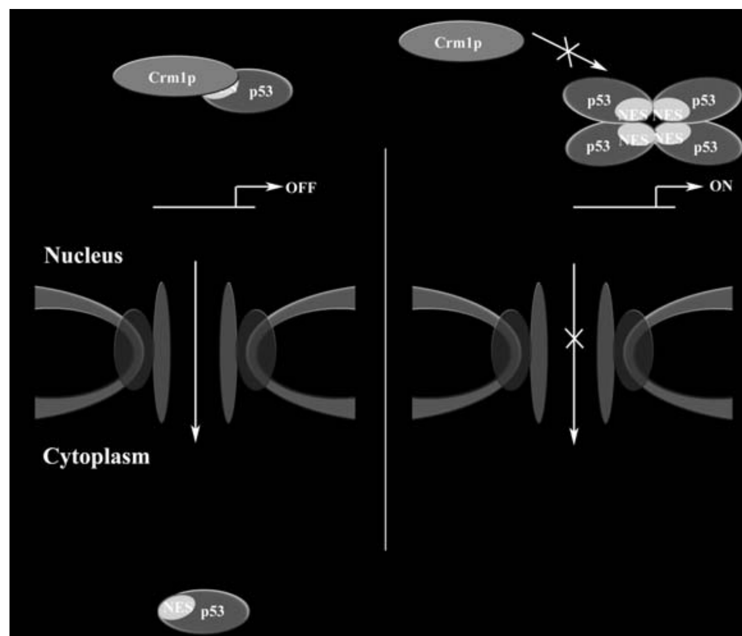

Figure 2. The regulation of p53 localization. The export karyopherin Crm1p recognizes a nuclear export signal (NES) on p53 and actively maintains p53 in the cytoplasm. When cells are stressed, p53 tetramerizes which enhances its ability to activate transcription (represented by the "ON" state of the diagrammed promoter). The tetramerization of p53 masks its NES, blocking its interaction with Crm1p and its export from the nucleus.

patterns of certain nup genes and their possible ability to gear nuclear transport to a specific tissues needs. Recent work has detailed differences in the expression of gp210 and Nup50 in different tissues (Olsson et al., 2004; Smitherman et al., 2000). In addition, the Drosophila homolog of Nup88, members only (mbo), is differentially expressed during larval development in specific cell types (Uv et al., 2000). Interestingly, mbo mutants exhibited developmental defects that were linked to tissue-specific import defects. These data suggest that tissues that lack mbo expression have NPCs that are structurally and functionally distinct and that they play key roles in larval development. Importantly, mbo mutants were also shown to have nuclear import defects for only certain NLS-bearing proteins, suggesting that mbo plays a role in controlling specific transport pathways (Uv et al., 2000).

The idea that individual nups can influence specific transport pathways has been developed in both yeast and vertebrates. During poliovirus infection, for example, distinct nuclear import pathways are inhibited. This effect is thought to be mediated by the selective degradation of two nups, Nup153 and p62, during viral infection (Gustin and Sarnow, 2001). Similarly, during vesicular stomatitis virus infection, specific nuclear transport pathways are also inhibited (Her et al., 1997; Peterson et al., 2000). In this case, transport inhibition is thought to be a direct consequence of the inhibitory effects of the viral M-protein and its interaction with Nup98 (von Kobbe et al., 2000). Importantly, these effects are supported by a number of studies evaluating the role that Nup98 and Nup153 play in regulating distinct nuclear transport pathways. The inhibition of Nup98 in Xenopus oocytes by injection of anti-Nup98 antibodies results in the specific inhibition of snRNA, mRNA and rRNA export but does not affect the export of tRNA (Powers et al., 1997). Similar experiments using antibodies against Nup153 also have no effect on tRNA export, but clearly inhibit the export of snRNA, mRNA and 5sRNA (Ullman et al., 1999). Perturbations of nuclear import pathways have also been observed. The depletion of Nup153 from Xenopus extracts results in the specific disruption of Kap-ß1/Kap-a mediated import, but the Kap-ß2 transport pathway is unaffected (Walther et al., 2001). Furthermore, the overexpression of dominant negative truncations of Nup153 can have specific effects on both the Kap-ß1 and Kap-ß2 import pathways (Shah and Forbes, 1998).

One logical assumption to explain these results is that there are unique pathways traveled by karyopherins as they move through the NPC that involve their binding to specific nups. The first of these binding sites was initially revealed between the yeast proteins Nup53p and Kap121p (Marelli et al., 1998), but has since been shown to include other kap/nup pairs. For example, in yeast, Kap95p has a specific binding site on Nup1p (Pyhtila and Rexach, 2003; Gilchrist and Rexach, 2003), and Nup2p has a specific binding site for Kap60p (Booth et al., 1999; Hood et al., 2000; Solsbacher et al., 2000; Matsuura et al., 2003; Gilchrist and Rexach, 2003). In vertebrates, specific binding sites have been mapped on Nup153 and Nup98 (Nakielny et al., 1999; Fontoura et al., 2000; Shah et al., 1998).

A common feature of these binding sites is that they are devoid of FGs and, in cases where it has been measured, they have affinities for kaps that are much stronger than the Kap-FG-repeat interaction (Pyhtila and Rexach, 2003; Matsuura et al., 2003; Ribbeck and Gorlich, 2001). Importantly, there is evidence linking these sites to the regulation of distinct nuclear import pathways (Gilchrist and Rexach, 2003; Pyhtila and Rexach, 2003; Matsuura et al., 2003; Makhnevych et al., 2003). The deletion of the specific binding site in Nup1p, for example, lowers the binding affinity for Kap95p 450-fold and has specific effects on Kap95p/Kap60p mediated import (Pyhtila and Rexach, 2003). Similarly, the mutation of the Kap60p binding site on Nup2p affects the efficiency of NLS import (Gilchrist and Rexach, 2003; Matsuura et al., 2003).

Another common feature of these binding sites is that, with the exception of Nup53p, they appear to cluster on the nuclear basket (Marelli et al., 1998; Rout et al., 2000; Cronshaw et al., 2002). The significance of this distribution may reflect a role for

these sites in promoting the dissociation of cargo from karyopherins and thus an aid in the termination step of specific import reactions (Fontoura et al., 2000; Gilchrist and Rexach, 2003; Matsuura et al., 2003). This idea has been developed in detail with respect to the Kap60p/Nup2p interaction, and has culminated with the elucidation of the crystal structure of Kap60p in complex with the high affinity Nup2p binding site (Matsuura et al., 2003). The structure revealed that the Nup2p peptide bound to a region of Kap60p that partially overlapped the NLS-binding groove. Since Kap60p binds Nup2p with a higher affinity than NLSs, this interaction is thought to displace the NLS. This observation has been independently confirmed biochemically by other groups (Solsbacher et al., 2000; Gilchrist and Rexach, 2003).

Perhaps another reason that these binding sites cluster on the nuclear side of the NPC, is that based on the transport models described earlier, high affinity kap binding sites within the NPC could function to inhibit nuclear import. Therefore, the symmetrical distribution of Nup53p on both the nuclear and cytoplasmic sides of the NPC would, superficially, be incompatible with current transport models. Yeast, however, have devised an elegant mechanism for sequestering and exposing Nup53p at specific stages of the cell cycle as a means of regulating Kap121p-mediated transport (Makhnevych et al., 2003). Kap121p interacts directly with Nup53p through an NLS-like site referred to as the KBD (Kap-Binding Domain)(Lusk et al., 2002). During interphase, Nup53p is bound to a neighbor nup, Nup170p, which interacts with a region of Nup53p that overlaps the KBD, thereby masking it from Kap121p. Upon entry into M-phase Nup53p is phosphorylated and there are discrete molecular rearrangements within the NPC that break the interaction between Nup53p and Nup170p, exposing the KBD to Kap121p. The binding of Kap121p to Nup53p inhibits its import into the nucleus and likely causes the premature release of its cargo. The result is a striking inhibition of the nuclear accumulation of Kap121p cargos during mitosis (Fig. 3). While it has yet to be determined which Kap121p cargos are affected by this pathway, constitutive activation of this inhibitory pathway by overexpression of NUP53 leads to a delay in mitotic progression. Importantly, this work is the first to describe a transport inhibitory function for a nup and further establishes a more elaborate role for the NPC in mediating nucleocytoplasmic transport than previously appreciated (Makhnevych et al., 2003).

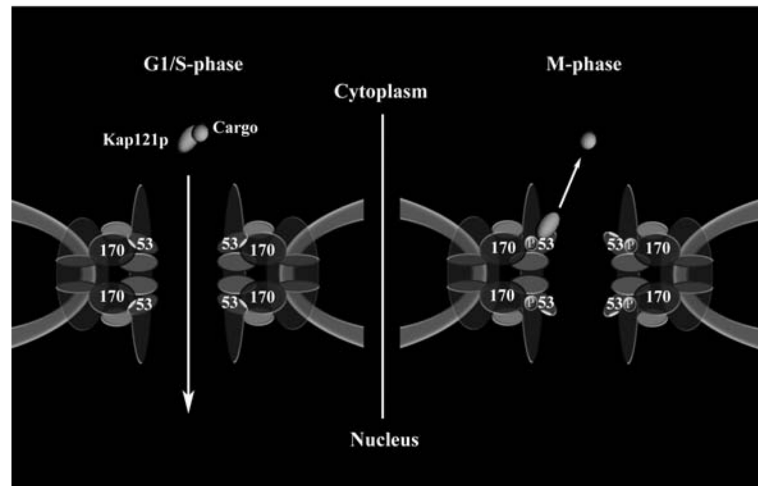

Figure 3. Regulating the Kap121p-mediated transport pathway. The interaction of Nup53p (53) with a neighbor nucleoporin, Nup170p (170), is altered between G1/S and M-phase of the cell cycle. The binding of Nup53p to Nup170p during G1/S masks a high affinity Kap121p binding domain (diagrammed as a yellow oval on Nup53p) and allows Kap121p import to procede unimpeded. In M-phase, Nup53p is phosphorylated (P) and the interaction between Nup53p and Nup170p is broken, exposing the Kap121p binding domain. The interaction between Kap121p and Nup53p inhibits Kap121p import and may induce the premature release of its cargo in the cytoplasm.

It is unlikely that this mechanism is unique to yeast. Recent functional links have been established between the phosphorylation of nups in Aspergillus nidulans and progression through the cell cycle (De Souza et al., 2003). The phosphorylation of Nup98 and Gle2 by NIMA kinase is necessary for the nuclear accumulation of both the mitotic kinase cdc2/cyclin B complex and tubulin in the nucleus. These events are essential for the timely progression into mitosis and the formation of the mitotic spindle (De Souza et al., 2003; Ovechkina et al., 2003). Furthermore, similar mechanisms likely function in organisms with an open mitosis at points prior to NE disassembly or shortly after its reformation, as well as at other points in the cell cycle. This intriguing idea is stimulated by previous observations in mammalian cells showing that a subset of nups, including

Nup153, Nup214, and Nup358, are phosphorylated during S-phase when the NE is intact (Favreau et al., 1996), as well as data showing that phosphorylation, most likely of nups, can inhibit nuclear transport (Kehlenbach and Gerace, 2000).

Conclusions

While it is clear that our current inventory of the components that make-up the transport machinery is nearly complete, our understanding of how they functionally interact to control transport continues to evolve. As this review details, this is particularly true with respect to the role of the NPC in modulating its molecular architecture to regulate its association with the soluble transport machinery. The capacity of the NPC to regulate nuclear transport of specific karyopherins and their cargos, or to globally alter levels of transport is indicative of both the fidelity and range of this transport system. The challenge ahead is to define the nuances of interactions that define these regulatory mechanisms, and how they impinge on cellular function.

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