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Review

Regulating Access to the Genome: Nucleocytoplasmic Transport throughout the Cell Cycle

Karsten Weis*
Department of Molecular and Cell Biology
Division of Cell and Developmental Biology
University of California, Berkeley
Berkeley, California 94720

Macromolecular transport between the cytoplasm and the nucleus occurs through the nuclear pore complex (NPC) and is mediated by multiple families of soluble transport factors. All these transport factors share the ability to translocate across the NPC through specific interactions with components of the nuclear pore. This review highlights advances in our understanding of the structure and function of the NPC and the shuttling transport receptors involved in nuclear transport. It discusses recently proposed models for the translocation of receptor-cargo complexes through the NPC channel and reviews how the small GTPase Ran functions as a positional marker of the genome to regulate multiple important aspects of the eukaryotic cell cycle.

Introduction

Transport of macromolecules between the nucleus and the cytoplasm is an essential cellular process in all eukaryotes. An astounding number of cargoes are hauled between these two compartments, consuming a considerable amount of the cell's energy pool. In return, this compartmentalization provides eukaryotic cells additional possibilities to regulate fundamental processes such as gene expression and signal transduction. Nucleocytoplasmic transport occurs through the nuclear pore complex (NPC), one of the biggest macromolecular assemblies in an eukaryotic cell. The NPC penetrates the two lipid bilayers of the nuclear envelope (NE) and can accommodate a large number of diverse RNA and protein cargoes, ranging in mass from a few kDa to almost 50 MDa (or almost 40 nm in diameter [Pante and Kann, 2002; reviewed in Rout and Aitchison, 2001; Vasu and Forbes, 2001]). Compared to most other transmembrane transporters, the NPC possesses at least two peculiar features. First, the NPC has to mediate efficient macromolecular transport in two directions and, second, translocation through the NPC does not require unfolding of the transported cargoes. The NPC contains an aqueous channel that permits the relatively unrestrained passage of macromolecules of up to 40–60 kDa (or up to 9 nm in diameter), and small proteins and metabolites can freely equilibrate between the nucleus and the cytoplasm. Yet, the NPC functions also as a highly selective molecular sieve, and macromolecules that are larger than 40–60 kDa in mass can only pass the NPC by active transport (Mattaj and Englmeier, 1998; Görlich and Kutay, 1999; Rout and Aitchison, 2001; Vasu and Forbes, 2001).

Most transport events through the NPC are mediated by soluble receptors that specifically recognize their cargoes and facilitate the passage of receptor-substrate complexes. As expected from the large variety of transport substrates, multiple classes of nuclear transport receptors exist. The biggest class is the family of importin β like transport factors (the importins/exportins, also named karyopherins). A typical metazoan cell probably expresses more than 20 members of this family and, as their names imply, importins or exportins can mediate either the nuclear import or export (and sometimes even both) of a very diverse set of protein or RNA cargoes (Mattaj and Englmeier, 1998; Görlich and Kutay, 1999; Macara, 2001; Ström and Weis, 2001; Lei and Silver, 2002; Weis, 2002). Members of this family are responsible for the recognition of the great majority of nuclear transport cargoes. The second class is represented by the small nuclear transport factor 2 (NTF2)/p10, which imports the small GTPase Ran into the nucleus (Ribbeck et al., 1998; Smith et al., 1998). Although NTF2 appears to transport only a single cargo, this represents one of the most impressive nuclear trafficking events numerically since several million molecules of Ran have to be imported every minute into the nucleus of an actively dividing mammalian cell (Mattaj and Englmeier, 1998; Görlich and Kutay, 1999). The third transport receptor family is involved in the nuclear export of mRNA. This mRNA exporter is a heterodimer of a large, conserved subunit named Mex67 in yeast or TAP/NXF in metazoans and a small subunit termed Mtr2 in yeast and p15/NXT in metazoans (Conti and Izaurralde, 2001; Reed and Hurt, 2002).

Interestingly, these three nuclear receptor classes have evolved independently and do not display any significant sequence homology. However, they operate via similar mechanisms, as they all share the ability to shuttle between the nucleus and the cytoplasm and interact with components of the nuclear pore that contain characteristic phenylalanine/glycine (FG)-rich repeat motifs.

Regulation of Transport Cargo Binding and Release: The RanGTP Gradient in Interphase

The regulation of cargo binding to and release from shuttling transport receptors is key to understanding nuclear transport. A transport receptor must specifically recognize its cargo in the originating compartment and then unload it at the target destination on the opposite side of the NPC. Upon delivery, empty receptors recycle back to undergo additional rounds of transport. Since transport can occur against a concentration gradient, this transport cycle must also be coupled to the consumption of energy.

How this regulation occurs is best understood for transport events mediated by the family of importins and exportins, which are regulated by the small GTPase Ran (Mattaj and Englmeier, 1998; Görlich and Kutay, 1999; Macara, 2001; Lei and Silver, 2002; Weis, 2002). As illustrated in Figure 1, substrate binding and release of importins and exportins is regulated by the asymmetric distribution of the two nucleotide states of Ran, the so-called RanGTP gradient. Like other small GTPases,

*Correspondence: kweis@uclink4.berkeley.edu

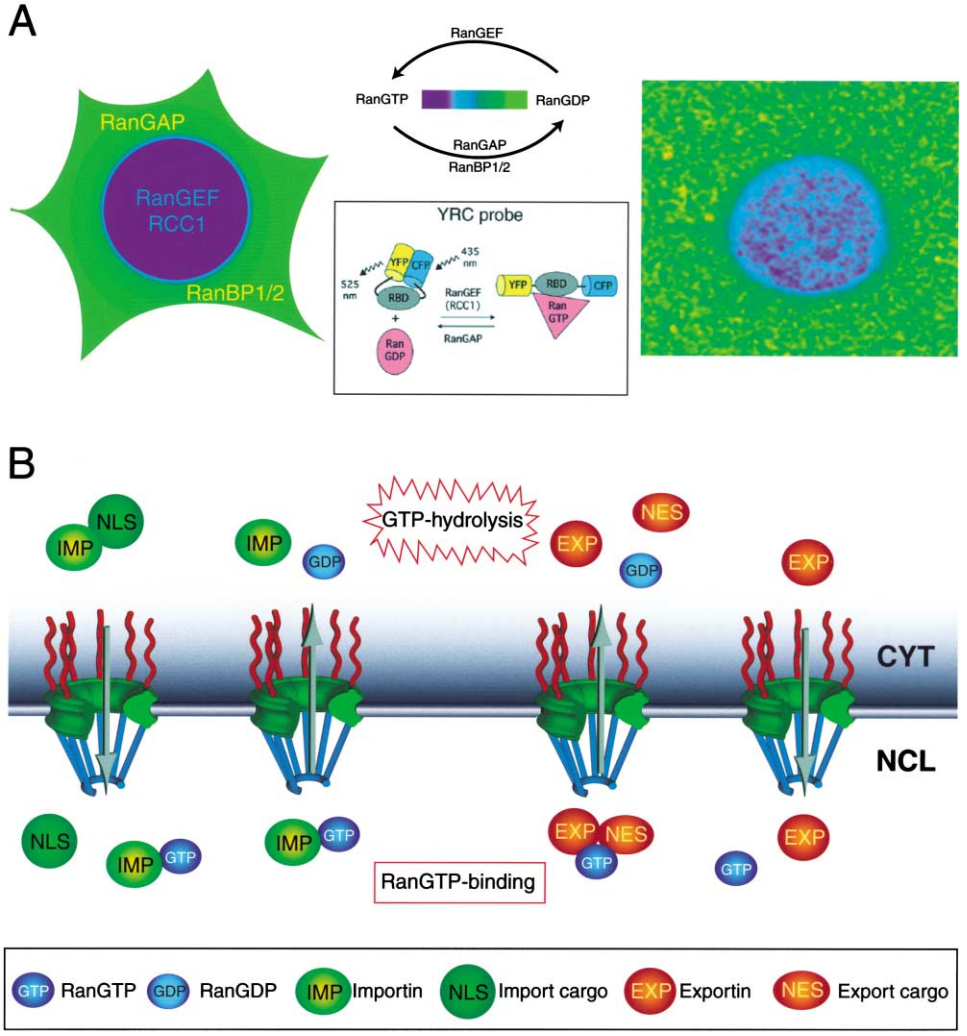

Figure 1. A Gradient of RanGTP Confers Directionality to Nuclear Transport

(A) The compartmentalized distribution of the RanGEF RCC1 in the nucleus and the RanGAP (together with the accessory proteins RanBP1/2) in the cytoplasm leads to a high enrichment of RanGTP in the nucleus. The gradient of RanGTP distribution can be visualized using FRET-based Ran biosensors (Kalab et al., 2002). The YRC sensor is a fusion between a RanGTP binding domain and the yellow and cyan fluorescent proteins. While no FRET occurs in the presence of RanGTP, YRC undergoes efficient FRET in the presence of RanGDP (or in the absence of Ran). A loss of FRET is displayed in dark blue, whereas the increase in FRET is shown in green in the pseudocolored ratio image (right panel). (B) Ran confers directionality to nuclear transport. Importins bind to their cargo in the cytoplasm and release their load upon RanGTP binding in the nucleus. The importin-RanGTP complex recycles back to the cytoplasm where GTP hydrolysis terminates the cycle. The free importin is then able to undergo additional rounds of transport. Exportins bind to their cargo in the nucleus in the presence of RanGTP. In the cytoplasm, GTP hydrolysis causes disassembly of the export complex and recycling of the export receptor (for details, see text).

Ran cycles between a GTP- and GDP bound state; however, the conversion between these two forms occurs slowly in the absence of accessory factors. Loading of GTP is catalyzed by the Ran guanine nucleotide exchange factor (RanGEF) RCC1, whereas RanGTP hydrolysis is stimulated by several orders of magnitude by the RanGTPase activating protein RanGAP together with the acessory proteins RanBP1 and RanBP2. The critical feature of the RanGTPase cycle is that GTP loading and GTP hydrolysis occur in different compartments (Figure 1; reviewed in Mattaj and Englmeier, 1998; Görlich and Kutay, 1999; Macara, 2001; Weis, 2002). Ran's GEF RCC1 is a very abundant protein that has been estimated to be present in up to one copy per nucleosome (Dasso et al., 1992). It is confined to the nucleus, where it directly binds to nucleosomes through an interaction with the core of histones H2A and H2B (Nemergut et al., 2001). In contrast, RanGAP and RanBP1/2 are restricted to the cytoplasm. In metazoan cells, RanGAP is found at its highest concentration at the outer face of the NPC, where it associates with the nucleoporin RanBP2 via its SUMO modification (Matunis et al., 1996; Mahajan et al., 1997). The highly compartmentalized distribution of the Ran regulators predicts a steep concentration difference of the two Ran nucleotide states between the nucleus and the cytoplasm, so that every molecule of Ran entering the nucleus is rapidly converted to the GTP-bound state and, conversely, RanGDP

predominates in the cytoplasm (Figure 1A; Mattaj and Englmeier, 1998; Görlich and Kutay, 1999; Macara, 2001; Weis, 2002). The unequal distribution of the two Ran nucleotide states provides important positional information to transport receptors of the importin/exportin family and—as discussed in more detail below—is necessary and sufficient for directional movement through the NPC.

Both importins and exportins interact with RanGTP through their conserved amino-terminal domains, yet RanGTP binding has the opposite effect on import and export receptors (Figure 1B). Importins bind their substrates in the absence of Ran in the cytoplasm and release them upon RanGTP binding in the nucleus. The cargo-free importin-RanGTP complex then rapidly recycles back to the cytoplasm. In contrast, exportins can bind to their cargo only in the presence of RanGTP and thus associate with their substrates exclusively in the nucleus. Upon export to the cytoplasm, the trimeric exportin/RanGTP/cargo complex is disassembled by RanGTP hydrolysis induced by RanGAP and RanBP1/2 (Figure 1B; reviewed in Mattaj and Englmeier, 1998; Görlich and Kutay, 1999; Macara, 2001; Weis, 2002).

For every transport cycle, at least one molecule of RanGTP is depleted from the nucleus, and the nuclear Ran pool needs to be replenished continuously. This is accomplished by the Ran importer NTF2 that very efficiently transports RanGDP from the cytoplasm to the nucleus (Ribbeck et al., 1998; Smith et al., 1998; Ribbeck and Gorlich, 2001). Cargo release and vectoriality for this transport reaction is again imparted by nucleotide exchange on Ran in the nucleus, which leads to the dissociation of RanGTP from NTF2.

The cornerstone of this model is the establishment and maintenance of a steep concentration difference of RanGTP and RanGDP across the nuclear envelope. In silico simulations estimated a nuclear to cytoplasmic RanGTP ratio of approximately 500 (Smith et al., 2002). Several in vivo and in vitro experiments strongly supported the necessity of a Ran gradient for directional nucleocytoplasmic transport (reviewed in Görlich and Kutay, 1999; Mattaj and Englmeier, 1998); yet, until recently, no direct evidence for its existence was available. However, new fluorescence resonance energy transfer (FRET)-based biosensors confirmed the hypothesis that a RanGTP gradient is indeed present in interphase nuclei (Figure 1A; Kalab et al., 2002). These Ran probes were developed to respond either directly or indirectly to the presence of RanGTP and have allowed the visualization of the two nucleotide states of Ran. Based on the Ran biosensor data, the concentration difference between free nuclear and cytoplasmic RanGTP was estimated to be at least 200-fold (Kalab et al., 2002).

In contrast to the rather complete picture for the transport cycle of importin β like proteins NTF2 and Ran, less is known how cargo binding and release is regulated for the third class of nuclear transport receptors, the mRNA exporter TAP/Mex67. To date, there is no evidence for a direct role of the Ran system in the TAP/Mex67 pathway, and the mechanism providing directionality to mRNA export remains unknown. However, much has been learned about the complex pathway that leads to the assembly of the mRNA export machinery onto the maturing mRNA in the nucleus. This topic has been the subject of several recent reviews, and the reader is referred to these for details and references (Cole, 2001; Conti and Izaurralde, 2001; Reed and Magni, 2001; Dreyfuss et al., 2002; Lei and Silver, 2002; Reed and Hurt, 2002; Weis, 2002). Overall, it has become apparent that mRNA export is tightly coupled to several steps of gene expression including transcriptional elongation, polyadenylation, and splicing. In contrast, it is still poorly understood how the exported mRNP is disassembled in the cytoplasm to allow recycling of TAP/Mex67 and other transport factors. Good candidates for mRNP remodeling factors include the cytoplasmic DEAD-box helicase Dbp5 (Snay-Hodge et al., 1998; Tseng et al., 1998; Schmitt et al., 1999) and the translating ribosome (Dostie and Dreyfuss, 2002). The detailed characterization of this important mRNP disassembly step remains one of the challenges in the field.

Importins and Exportins: The Details

Members of the importin β family (named importins/exportins or karyopherins) are accountable for the recognition of the majority of nuclear import and export cargoes. More than 20 members of this family have been identified in metazoans (Görlich and Kutay, 1999; Ström and Weis, 2001). For ten of these (Importin β, transportin 1, transportin SR, Imp4, Imp5, Imp7, Imp8, Imp9, Imp11, and Imp13), a role in nuclear import has been identified, whereas six were shown to function in nuclear export (Crm1, CAS, exportin-t, Exp4, Exp5, and Imp13). The *S. cerevisiae* genome encodes for 14 importins/exportins, of which ten have a characterized role in import (Kap95, Kap104, Kap108, Mtr10/Kap111, Kap114, Nmd5/Kap119, Pse1/Kap121, Pdr6/Kap122, Kap123, Msn5/Kap142) and four function in export (Crm1/Xpo1, Cse1, Los1, and Msn5/Kap142). A major effort in the field has been directed toward the identification of cargoes for individual members of the importin β family (for a review and a list of cargoes see Ström and Weis, 2001). This has led to the identification of bona fide import or export substrates for most of the factors listed above and unveiled a significant redundancy between certain transport pathways. For example, in vitro import assays in mammalian cells revealed that five different importins can mediate the import of histones (Muhlhausser et al., 2001), and at least four importin β like factors are able to transport ribosomal proteins into the nucleus (Jäkel and Görlich, 1998). Unfortunately, with the exception of a few cases, the cargo recognition sites have not been mapped in detail. Although these interactions are generally thought to be mediated by small, transferable signal sequences, only a small number of such consensus binding sites have been identified so far. The best studied examples are the lysine-rich "classical" nuclear localization sequence (NLS) originally identified in the SV40 T antigen (Kalderon et al., 1984; Lanford and Butel, 1984), which is recognized by the importin α/β dimer (reviewed in Görlich and Kutay, 1999; Mattaj and Englmeier, 1998) and the leucine-rich nuclear export sequence (NES), originally identified in HIV Rev and PKI (Fischer et al., 1995; Wen et al., 1995), recognized by Crm1 (Mattaj and Englmeier, 1998; Görlich and Kutay, 1999). Thus, despite the impressive number of receptor-cargo interactions that have been studied, the prediction

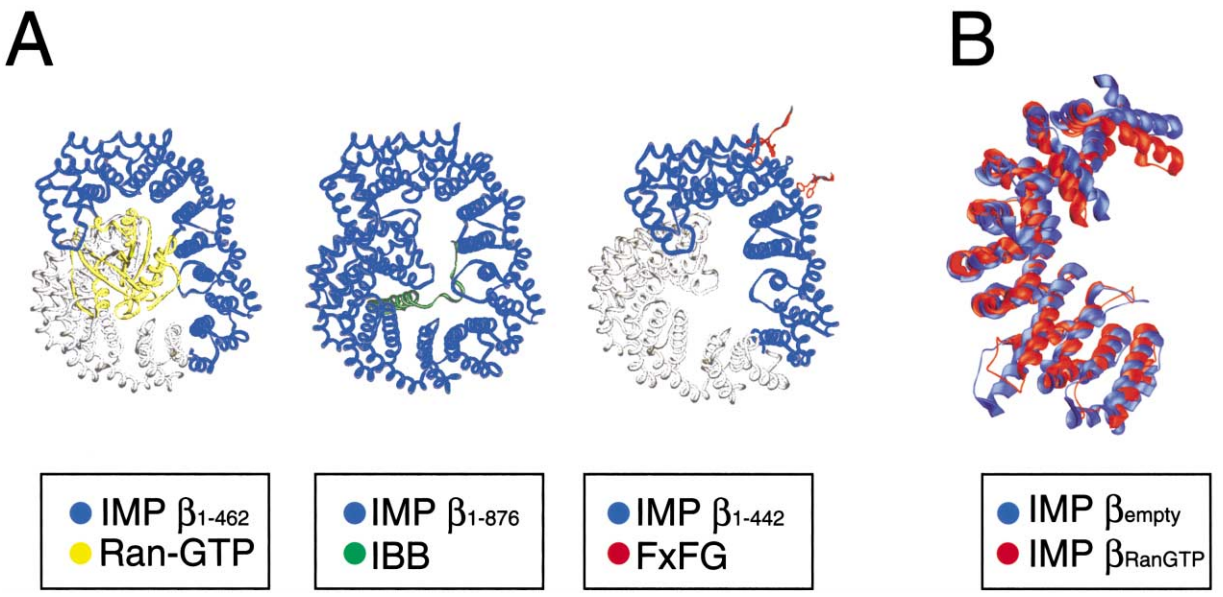

Figure 2. Structure of Importin β

(A) Structural alignment of importin β (1-462) bound to Ran (Vetter et al., 1999), full-length importin β bound to the IBB fragment of importin α (Cingolani et al., 1999), and importin β (1-442) bound to FXFG-containing peptides (Bayliss et al., 2000). While Ran and IBB cargo bind to the concave surface of importin β, the interaction with components of the NPC occurs on the convex face of the superhelix. The structure of importin β is shown in blue and the hypothetical trace of the carboxy-terminal residues missing in the Ran- and FXFG bound structures are modeled in gray.

(B) The amino-terminal structure of the empty (blue) and the Ran bound (red) form of importin β are superimposed to illustrate the conformational changes in the two forms. Importin β is flexible, and binding to RanGTP induces a shift in the structural arrangement of the HEAT repeats.

of nuclear localization and nuclear export signals in candidate proteins remains extremely difficult.

To fully explain the biochemistry of Ran-regulated substrate binding and release, a detailed knowledge of the importin/exportin protein structure is necessary. Unfortunately, no three-dimensional structural information is currently available for the exportin subclass of transport receptors, and very little is known about the basis of their RanGTP binding requirement for substrate recognition. However, crystal structures of importin β and transportin 1 (also known as karyopherin β2) were solved either in a complex with a cargo (Cingolani et al., 1999), with RanGTP (Chook and Blobel, 1999; Vetter et al., 1999), with an FG-repeat peptide (Bayliss et al., 2000), or in a free form (Lee et al., 2000). Importin β and transportin 1 have a similar overall architecture and consist of multiple HEAT repeats, arranged to form a snail-like superhelical structure (Figure 2). Cargo and Ran binding surfaces are oriented toward the interior of the superhelix, whereas the interactions with the FG repeats occur on the outer surface of the helical structure (Figure 2A). Interestingly, the structure of the uncomplexed importin β structure reveals a different superhelical twist than the RanGTP bound structure, suggesting that importin β undergoes relatively large conformational changes in HEAT repeat helix stacking upon RanGTP binding (Figure 2B; Lee et al., 2000). Although the sequence homology is limited, it is predicted that exportins fold in a similar way as importin β and undergo comparable conformational changes upon RanGTP binding. Therefore, it is conceivable that such RanGTP-induced conformational changes are necessary for the creation of export cargo binding surfaces.

All transport receptors of the importin/exportin family can bind to at least some targets directly (either alone or in a complex with RanGTP). As illustrated in Figure 1, a single round of RanGTP hydrolysis is expended to transport one cargo unidirectionally across the nuclear envelope in this direct binding mode. However, it has also become apparent that some transporters are able to carry cargoes bidirectionally across the nuclear envelope. For example, distinct import and export cargoes have been identified for both importin 13 in mammals (Mingot et al., 2001) and Msn5 in yeast (Yoshida and Blobel, 2001). This mode of transportation obviously increases the efficiency, since these carriers allow the transport of two substrates in opposite directions per RanGTP hydrolysis cycle. In addition to the direct binding mode, some importins or exportins also use adaptor proteins to interact with their cargo. This has been best studied for the import receptor for classical nuclear localization signal (NLS)-containing proteins, consisting of the importin β/importin α dimer. Importin α recognizes the cargo NLS but requires importin β to cross the NPC. Upon cargo delivery, importin β returns to the cytoplasm presumably in complex with RanGTP, but importin α additionally requires the exportin CAS (and RanGTP) for its recycling (reviewed in Mattaj and Englmeier, 1998; Görlich and Kutay, 1999). Therefore, at least two GTP molecules are consumed per NLS import cycle. Even more complex is the export of snRNAs, which use the adaptors PHAX and CBC in order to bridge the interaction between snRNAs and the exportin Crm1 (Ohno et al., 2000). Furthermore, PHAX is subject to a reversible phosphorylation cycle, which is necessary to complete snRNA export (Ohno et al., 2000). Although energetically

less efficient, the use of adapters can significantly broaden the cargo choice (as evident for importin α/β), while the additional expenditure of energy introduces additional possibilities to regulate transport.

Another complication to the Ran paradigm is the use of accessory factors that are regulators of cargo binding or release. For certain cargoes, it was known that the Ran system alone is not sufficient to regulate the association/dissociation cycle. For example, the yeast RNA binding protein Npl3 only dissociates from its importin Mtr10 in the presence of RanGTP and RNA (Senger et al., 1998). A more general cofactor is the RanGTP binding protein RanBP3, which was recently shown to function in the exportin1/Crm1 pathway. RanBP3 displays homology to the cytoplasmic dissociation factors RanBP1 and 2 (see Figure 1), but because of its nuclear localization, its detailed function had been unclear. Recently, it was demonstrated that RanBP3 increases the affinity of Crm1 for its cargo and stimulates its export in vitro (Englmeier et al., 2001; Lindsay et al., 2001). Another cofactor for nucleocytoplasmic transport is Nup50/Npap60, which was shown to stimulate the import of classical NLS cargoes. Nup50/Npap60 has multiple binding surfaces and functions in a switch-like fashion, forming distinct complexes with RanGTP, importin α, and importin β (Lindsay et al., 2002). Neither RanBP3 nor Nup50/Npap60 are absolutely required for transport, but they increase the efficiency of the trafficking event. As discussed in more detail below, cargo binding and release must be tightly regulated on the opposite faces of the NPC to prevent retrograde movements of the cargo receptor complexes, and additional cofactors may be critical to spatially and temporally coordinate these events.

The Nuclear Pore Complex: Composition and Structure

The nuclear pore compelx (NPC) is one of the biggest macromolecular structures in a eukaryotic cell, varying in mass between $\sim$50 MDa in yeast and $\sim$125 MDa in vertebrates (Fahrenkrog et al., 2001; Rout and Aitchison, 2001; Vasu and Forbes, 2001). Despite the rather large difference in mass, the overall architecture of the NPC appears to be well conserved between species. Using electron microscopy, three-dimensional NPC structures have been generated based on nuclear pore preparations from amphibian oocytes (Hinshaw et al., 1992; Akey and Radermacher, 1993) and from *S. cerevisiae* (Yang et al., 1998). These low-resolution structures reveal that the core of the NPC consists of a cylinder with 8-fold rotational symmetry, which spans the nuclear envelope and encircles a central channel (Figure 3A). Additional peripheral filaments emanate from each spoke of the cylinder, pointing toward the nucleus and the cytoplasm. These peripheral structures are not present in the three-dimensional models but have been visualized by various electron microscopic techniques in the NPCs of different species (Fahrenkrog et al., 2001; Rout and Aitchison, 2001; Vasu and Forbes, 2001).

An important advance in our understanding of nuclear pore structure and function has been the biochemical and genetic dissection of its constituents, the nucleoporins (reviewed in Rout and Aitchison, 2001; Vasu and Forbes, 2001). Work from many laboratories culminated in the recent proteomic analyses of NPCs purified from yeast (Rout et al., 2000) and mammalian cells (Cronshaw et al., 2002). The detailed characterization of the NPC composition revealed several major surprises. The first surprise was the small number of distinct nucleoporins that can be found in NPC preparations. Despite its gigantic size, the NPC is made up of only $\sim$30 different nucleoporins in yeast and in mammals (for comparison, the 15–30 times smaller ribosome, which has a mass of 4 MDa consists of consists of 80 different proteins). Although both proteomic analyses did not include a functional criterion as the basis for their purification scheme, it is expected that the parts list presented in these studies is almost complete. This brings up the question of how such a massive structure can be constructed from so few distinct proteins? The answer appears to be the high copy number of individual nucleoporins within the NPC, owing to the symmetry of the nuclear pore structure. In yeast, most nucleoporins were estimated to be present in either 16 or 32 copies per NPC (Rout et al., 2000). The majority of the yeast nucleoporins are distributed symmetrically on both the cytoplasmic and nuclear faces of the pore and are therefore present in two or four copies in each of the eight spokes (Figure 3B). This was unexpected since it had been generally believed that the pore is highly asymmetric with respect to its cytoplasmic and nucleoplasmic organization.

Another surprise emerging from these studies is the relatively low sequence conservation of nuclear pore proteins between species. This is rather unusual for a structure of this size, since components of large protein complexes tend to participate in a large number of critical protein-protein interactions between subunits and are therefore highly conserved in sequence. Although domain organization and localization can be used to recognize potential orthologs between species, the sequence identity between these homologous proteins is generally lower than 20%–25% (Cronshaw et al., 2002). The low degree of sequence conservation seems to be incompatible with a translocation mode that would rely on highly coordinated structural rearrangements within the nuclear pore and thus would involve intricate interactions between multiple nucleoporins but is consistent with more simple models of facilitated transport discussed in detail below. Interestingly, the most highly conserved feature between yeast and mammalian nucleoporins is a FG dipeptide repeat motif present in approximately one third of all core components of the pore (Rout et al., 2000; Cronshaw et al., 2002). These FG repeats provide important interaction sites for transport factors and appear to play a central role in mediating the translocation of cargo-loaded receptors through the NPC.

The Nuclear Pore Complex: From Structure to Function

It appears that some of the most important biochemical principles that govern nucleocytoplasmic transport have been uncovered. The Ran paradigm provides an elegant explanation of how cargo recognition and release is regulated in a compartment-specific manner.

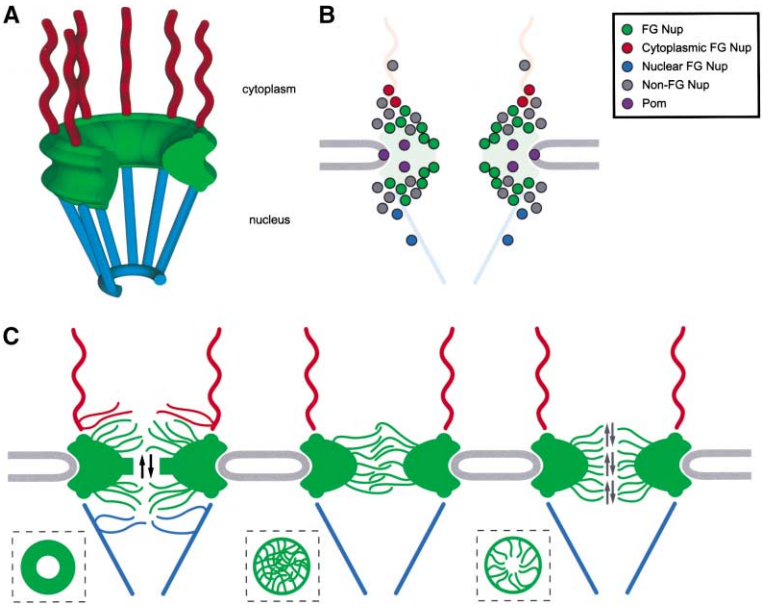

Figure 3. Models of Nuclear Pore Complex Structure and Function

(A) The nuclear pore complex (NPC) consists of eight spokes that form a cylinder embedded in the nuclear envelope. Several ring-like structures connect the spokes. Additional filamentous structures emanate from the NPC core and point toward the lumen of the nucleus and the cytoplasm.

(B) A model of the yeast NPC (adapted from Rout et al., 2000, reproduced from The Journal of Cell Biology, 2000,volume 148, 635–651, by copyright permission of The Rockefeller University Press) reveals that most FG-containing nucleporins (Nups) are symmetrically organized and can be found on both sites of the pore. Pore membrane proteins (POMs) are displayed in purple.

(C) Schematic illustrations of NPC selectivity models. The Brownian affinity gate model (Rout et al., 2000) proposes that the NPC channel consists of a narrow central tube. Binding to peripheral FG-repeat-containing nucleoporins increases the probability of entering the channel and thus facilitates the translocation step. Translocation itself occurs by Brownian motion (left panel). The selective phase model (Ribbeck and Gorlich, 2001) puts forward that the NPC channel represents a selective phase consisting of a meshwork formed by weakly interacting, hydrophobic FG-rich repeats. The selective phase can only be entered and permeated by transport receptors that can interact with FG-repeats and disrupt the meshwork (middle). The "oily-spaghetti" model (Macara, 2001) proposes that the open NPC channel is filled by hydrophobic, non-interacting FG-repeats that can be pushed aside by receptor-cargo complexes but prevent the passage of other molecules (right). The inserts show a cross-section through the central channel of the NPC according to the three models.

Furthermore, the specific interaction between various transport receptors and FG-rich nucleoporin domains present in a high copy number in the NPC have been well characterized. The biggest remaining problem is to understand the mechanistic details of how the NPC operates to achieve its exquisite selectivity and how it can mediate the large number of translocation events through its long central channel.

The energetics of nuclear transport have been studied in detail using in vitro transport assays. It was demonstrated that the metabolic energy, which is required to import an importin cargo into the nucleus, is exclusively supplied by the RanGTPase cycle (Weis et al., 1996). This is entirely consistent with the absence of any NTPases or motor-like activities in purifed NPCs (Rout et al., 2000; Cronshaw et al., 2002). However, Ran only acts to regulate cargo binding and release reactions, since NPC translocation itself does not require RanGTP hydrolysis (Kose et al., 1997; Nakielny and Dreyfuss, 1997; Schwoebel et al., 1998; Englmeier et al., 1999; Nachury and Weis, 1999; but see also Lyman et al., 2002), and the direction of transport can be inverted in the absence of the RanGTP gradient (Nachury and Weis, 1999). These results suggest that the NPC operates as a highly specialized channel, which selectively allows the facilitated transport of cargoes that are bound to transport receptors that share the ability to recognize FG-containing nucleoporins. According to this model, the translocation of receptor-cargo complexes through the nuclear pore is achieved by multiple low-affinity interactions (Chaillan-Huntington et al., 2000; Ribbeck and Gorlich, 2001), but no directionality is inherently built into these binding reactions. Instead, the vectorial nature of transport is ensured by irreversible steps on the two sites of the NPC (Nachury and Weis, 1999). These irreversible steps are best understood for transport events mediated by importin β like transport factors, which are terminated by RanGTP binding or GTP hydrolysis on the opposite faces of the NPC (Figure 1B).

It has also been proposed that an increase in affinities for transport factors along the NPC channel could contribute to the vectorial nature of transport (Ben-Efraim and Gerace, 2001). This is unlikely for several reasons. First, transport of both empty and cargo-loaded receptors is fully reversible in vitro and occurs at similar rate in both directions (Kose et al., 1997; Nakielny and Dreyfuss, 1997; Nachury and Weis, 1999). Second, the core of the NPC appears to be highly symmetric, and most nucleoporins can be detected on both sites of the pore (Rout et al., 2000). Third, the equilibrium process of binding and release of transport receptors to NPC components alone is unable to create order since it does not consume energy. However, the questions of why the pore has asymmetric fibrillar extensions and why high-affinity binding sites can be indeed detected at the ends of the pore (Allen et al., 2001; Ben-Efraim and Gerace, 2001) remain. It is interesting that nuclear pores, from which the cytoplasmic filaments have been experimentally removed, are able to mediate protein import in vitro (Walther et al., 2002). However, at least some of the high-affinity binding sites appear to be critical since transport receptor mutants that are irreversibly bound to these terminal sites are known to effectively inhibit transport through the pore (Kutay et al., 1997). It is conceivable that these high-affinity "docking sites" at the openings of the pore provide platforms to coordinate cargo binding and release with the recycling of the transport receptors. These terminal steps of transport have

to be tightly spatially controlled to prevent any retrograde flux of receptor cargo complexes. This is especially critical since net transport through the pore occurs against steep concentration gradients. To test this, it will be critical to map the exact position where substrate release and GTP hydrolysis occurs and to analyze the distribution of the Ran gradient within the pore.

The model of facilitated translocation does not address the problem of how the NPC achieves its high degree of selectivity for nuclear transport receptors and how it excludes non-signal-containing proteins. Several models have been put forward that in principle can account for the gating properties of the NPC (Figure 3C; Rout et al., 2000; Ribbeck and Gorlich, 2001; Macara, 2001). Rout et al. (2000) proposed a Brownian affinity gate model, which states that the aqueous NPC channel consists of a narrow central tube that is occluded by FG-rich filaments preventing passive diffusion. In this model, specific docking at FG-repeats facilitates the entry into the pore and then allows translocation by Brownian (i.e., random) motion (Rout et al., 2000). Based on kinetic data, Ribbeck and Görlich proposed a selective phase model for translocation through the NPC. This model proposes that the NPC channel is filled with a selective phase consisting of a meshwork formed by weakly interacting, hydrophobic FG-rich repeats. The FG-repeat meshwork functions as a sieve that can be dissolved by transient interactions with the translocating receptor-cargo complexes (Ribbeck and Gorlich, 2001). A very similar model suggests that the NPC channel is filled with unstructured, non-interacting FG-repeats or "oily-spaghetti" that can be pushed aside by receptor-cargo complexes but would hinder the passage of other molecules (Macara, 2001). Consistent with the energetic requirements for transport, all three models propose that NPC selectivity is exclusively achieved by conserved FG-repeat motifs that are located within the pore and that no active gating process is involved in the translocation step. Although these three models share several similarities, they are distinct in the details of how the FG repeats operate to achieve selectivity (Figure 3C). In support for the selective phase model, Ribbeck and Gorlich (2002) recently reported that the organic solvent cyclohexane-1,2-diol causes a specific increase in the permeability barrier of the NPC. This is consistent with the existence of a hydrophobic meshwork inside the channel, which is required to maintain the selectivity of the NPC. However, additional biophysical studies and, ultimately, a higher-resolution structure of the NPC will be required to unveil all the mechanistic details of the translocation event and to fully understand the principles of NPC function.

Cargo Delivery Outside Interphase: The Ran Gradient in Mitosis

It has long been suspected that the Ran cycle may have additional functions outside its well-established role in nucleocytoplasmic transport since mutations in components of the Ran cycle display pleiotropic phenotypes and often affect steps in cell division (discussed in Sazer and Dasso, 2000; Moore, 2001). However, the interpretation of these phenotypes has been inherently difficult because it could not be conclusively shown that they are direct effects rather than indirect consequences of a block in nuclear import or export. A key finding in support of the existence of additional mitotic functions of Ran was the observation that the addition of RanGTP to meiotically arrested *Xenopus* egg extracts (i.e., in the absence of nuclei) had dramatic effects on microtubule organization and induced the formation of microtubule asters (Kalab et al., 1999; Carazo-Salas et al., 1999; Ohba et al., 1999; Wilde and Zheng, 1999; Zhang et al., 1999). This result led to the proposal that RCC1-generated RanGTP stabilizes microtubules specifically in the vicinity of chromatin, thus providing an explanation for previous observations that chromatin influences microtubule dynamics and contributes to spindle assembly (Figure 4; Carazo-Salas et al., 1999; Kalab et al., 1999). Supporting the model that RanGTP acts locally to control microtubule nucleation and/or stabilization around chromosomes, the existence of a RanGTP gradient around mitotic chromosomes was demonstrated recently using nucleotide-specific Ran biosensors (Figure 4A; Kalab et al., 2002). Furthermore, it was shown that Ran itself is able to bind to histones H3 and H4 in vitro (Bilbao-Cortes et al., 2002) and to interact with chromatin in vivo (Hinkle et al., 2002). Therefore, Ran appears to function in mitosis and in interphase as a positional marker that delineates the perichromatin space (corresponding to the nucleoplasm in interphase).

Interestingly, the similarity to interphase transport does not end here since it was shown that the mitotic effects of Ran on microtubules are largely mediated by the nuclear transport factor importin β (Gruss et al., 2001; Nachury et al., 2001; Wiese et al., 2001). The two subunits of the NLS receptor, importin α and β, were shown to inhibit mitotic spindle assembly in vitro (Gruss et al., 2001; Nachury et al., 2001; Wiese et al., 2001) and in vivo (Nachury et al., 2001). It was proposed that the importin α/β dimer stoichiometrically sequesters and inhibits an aster-promoting activity (APA) and that this inhibitory effect is locally relieved by RanGTP inducing APA release around chromatin (Figure 4B). Two APA-like activities were identified as the microtubule-associated proteins (MAPs) TPX2 and NuMA (Gruss et al., 2001; Nachury et al., 2001; Wiese et al., 2001). Consistent with the model of a targeted delivery by importin α/β, both TPX2 and NuMA are nuclear in interphase. This localization also provides a potential regulatory mechanism to prevent access of these regulators of microtubule dynamics to interphase microtubules. Additional MAPs have been shown to localize to the nucleus in interphase and, thus, could be regulated in a similar manner in interphase and mitosis.

Growing evidence suggests a general role of Ran in the regulation of the mitotic microtubule network in vivo. RNA interference, microinjections, and mutants were used to demonstrate that the Ran system (Fleig et al., 2000; Guarguaglini et al., 2000; Bamba et al., 2002), importin β (Nachury et al., 2001), and TPX2 (Gruss et al., 2002) are required for spindle assembly in tissue culture cells in *C. elegans* and in *S. pombe*. From these and other studies, it has become obvious that the regulation of NuMA and TPX2 alone cannot account for all the phenotypes observed in cells with a disrupted Ran system. Currently, it cannot be fully explained how Ran increases microtubule stability through effects on changes in

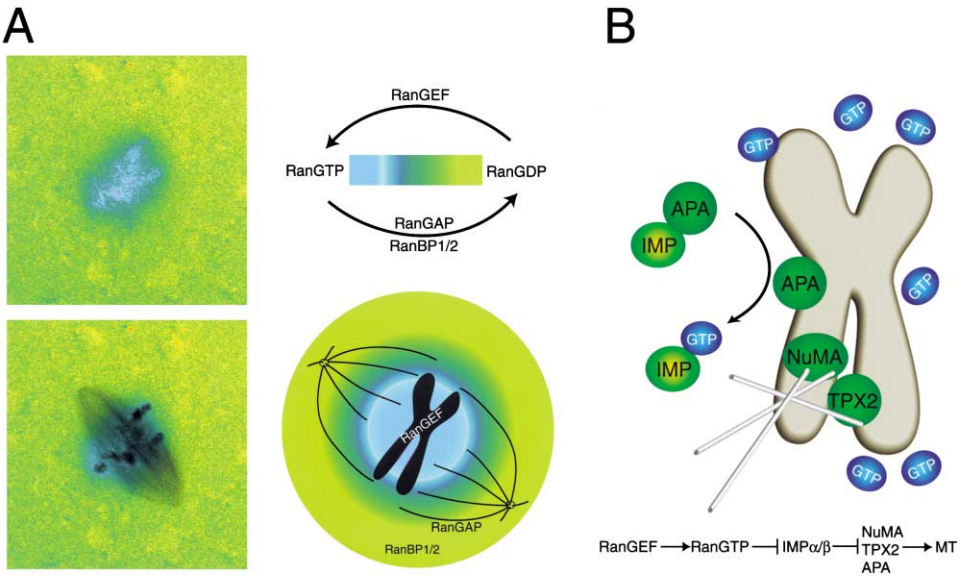

Figure 4. A Gradient of RanGTP around Chromatin Regulates Mitotic Spindle Assembly

(A) A gradient of RanGTP around mitotic chromatin can be visualized by Ran biosensors (Kalab et al., 2002). Ratio images using the YRC biosensor (Kalab et al., 2002; but see also Figure 1) demonstrate that RanGTP (displayed in light blue) is highly enriched in the vicinity of chromatin (top and bottom). The overlay image (bottom) illustrates the position of DNA (stained with Hoechst, displayed in black) and of fluorescent tubulin (displayed in dark gray). The schematic illustration indicates the position of the RanGEF RCC1 on chromatin and the localization of RanBP1/2 and RanGAP, which was shown to be enriched on the spindle and on kinetochores (Joseph et al., 2002; Matunis et al., 1996).

(B) Schematic illustration of the Ran microtubule pathway. RanGTP is specifically generated around chromatin by the DNA bound RanGEF RCC1. RanGTP binds to the transport factor importin β, inducing the release of cargoes (NuMA, TPX2, and APA) that function to regulate microtubules (see text for details).

growth rates and shrinkage frequency (Carazo-Salas et al., 2001; Wilde et al., 2001), how Ran regulates the motor protein Eg5 (Wilde et al., 2001), how Ran affects the nucleation capacity of the centrosome (Carazo-Salas et al., 2001), or how the centrosomal- and the Ran-mediated microtubule nucleation are coordinated in somatic cells. Finally, it is also not clear how the localization of RanGAP to the mitotic spindle (Matunis et al., 1996; Joseph et al., 2002) and to the kinetochores (Joseph et al., 2002) contributes to the Ran effects on microtubules or whether Ran influences microtubule attachment at the kinetochore. An important goal for the future remains to characterize the full scope of the Ran regulation and to elucidate the mechanistic details of how the Ran cascade influences mitotic spindle assembly.

Other Mitotic Functions of Ran: Ran and the Nuclear Envelope

In another twist to the story, the RanGTPase cycle has also been shown to play an important role at the end of mitosis, as Ran is required for nuclear envelope assembly (Hetzer et al., 2000; Zhang and Clarke, 2000). Intriguingly, it was demonstrated that Ran immobilized on beads is able to induce the formation of nuclear envelope-like structures. The structures that form around the Ran-coated beads contain NPCs and are able to mediate nuclear transport (Zhang and Clarke, 2000). Furthermore, depletion of either RCC1 or Ran specifically inhibits in vitro nuclear envelope formation at a very early step of the process (Hetzer et al., 2000).

How does Ran function in nuclear envelope formation? Consistent with the paradigm described above, Ran can be viewed again as positional marker signaling the position of the genome at the end of mitosis. The data described above suggest that Ran localization is critical and that Ran is both necessary and sufficient to direct nuclear envelope assembly around chromatin. However, it appears that there are mechanistic differences between the role of Ran in nuclear envelope formation and its function in nuclear transport and mitotic spindle assembly. First, nuclear envelope assembly requires both nucleotide exchange and GTP hydrolysis, and Ran in the GTP bound state alone is not "active" (Hetzer et al., 2000; Zhang and Clarke, 2000, 2001). Second, importin β was identified as a potential downstream effector of Ran in nuclear envelope formation, but its role in this pathway appears to be distinct from its function in spindle assembly (Zhang et al., 2002). Importin β does not require its cargo binding domain but instead uses its nucleoporin interaction surface to induce membrane formation around beads. From these experiments, it appears that Ran does not operate in nuclear envelope formation simply by regulating the localized release of substrates from transport factors. However, the mechanism remains unclear how Ran promotes membrane fusion or provides positional information to the topologically complex formation of a nuclear envelope. Also, it is not known whether Ran plays a direct role in the complex and poorly understood NPC assembly pathway. While it is intriguing that the NPC binding domain of importin β induces membrane formation around beads

(Zhang and Clarke, 2000) and that Ran itself is able to directly bind to chromatin (Bilbao-Cortes et al., 2002), it remains to be tested whether these interactions are physiologically important and how they contribute to the proper formation of a eukaryotic nucleus.

Ran Signals the Position of the Eukaryotic Genome

From the various functions described for the Ran-GTPase cycle, it is becoming clear that Ran is a master regulator of multiple aspects of nuclear physiology. A paradigm has emerged that implicates Ran as a positional marker, signaling the position of the eukaryotic genome throughout the cell cycle: in interphase, it flags the position of the nucleus and provides directionality to nucleocytoplasmic transport; in ana- and metaphase, it regulates microtubule nucleation around chromatin, and in telophase, Ran is required for nuclear envelope formation around chromatin. The involvement of Ran in so many important aspects of eukaryotic cell biology is intriguing and raises the question about the evolution and the conservation of this pathway. What aspects of Ran-regulated microtubule assembly are conserved in organisms that undergo a closed mitosis, and how has the Ran-signaling pathway been expanded in various species? From an evolutionary point of view, it could be speculated that the ancestral role of Ran was to promote the localized release of a spindle assembly factor in the proximity of chromatin and that the function of Ran in spindle assembly preceded its function in nuclear transport (or envelope assembly). After the evolution of the nuclear envelope, this role may have become more general to regulate transport events between the cytoplasm and the perichromatin space/nucleoplasm. However, it would not be completely unexpected if Ran has additional functions in other chromatin-centered events that have not been uncovered yet. Evidence already exists that the Ran system influences the process of chromatin condensation and affects the regulation of the cell cycle (reviewed in Moore, 2001; Sazer and Dasso, 2000). In the future, the Ran field may still be in for some surprises.

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