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Molecular Dissection of the Nuclear Pore Complex

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ABSTRACT: The nuclear pore complex (NPC) is a ~120 megadalton (MDa) supramolecular assembly embedded in the double-membraned nuclear envelope (NE) that mediates bidirectional molecular trafficking between the cytoplasm and the nucleus of interphase eukaryotic cells. The structure of the NPC has been studied extensively by electron microscopy (EM), and a consensus model of its basic framework has emerged. Over the past few years, there has been significant progress in dissecting the molecular constituents of the NPC and in identifying distinct NPC subcomplexes. The combination of well-characterized antibodies with different EM specimen preparation methods has allowed localization of several of these proteins within the three-dimensional (3-D) architecture of the NPC. Thus, the *molecular* dissection of the NPC is definitely on its way to being elucidated. Here, we review these findings and discuss the emerging structural concepts.

KEY WORDS: atomic force microscopy, electron microscopy, immuno-EM, nuclear envelope, nuclear pore complex, nucleoporin.

I. INTRODUCTION

In interphase eukaryotic cells, the nucleus is demarcated from the cytoplasm by a double-membrane system called the nuclear envelope (NE). As illustrated schematically in Plate 1, the NE consists of an inner and an outer nuclear membrane enclosing a lumen called the 'perinuclear space'. The NE enables the nucleus and the cytoplasm to have their distinctive composition, and it physically separates the genetic machinery from protein synthesis. In addition, the NE allows molecular trafficking between the cytoplasm and the nucleus: most of the RNA synthesized in the nucleus is exported to the cytoplasm, where it is used as a template for protein synthesis, while proteins required for nuclear function are synthesized in the cytoplasm and imported into the nucleus. This bidirectional exchange of material between the nucleus and the cytoplasm is crucial for the survival

* Plate 1 appears following page 184.

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of the cell, and it is mediated by large supramolecular assemblies embedded in the NE called nuclear pore complexes (NPCs). As indicated in Plate 1, the NPCs allow passive diffusion of ions and small molecules, and mediate active transport of nuclear proteins, RNAs, and ribonucleoprotein (RNP) particles. Whereas the first process occurs through aqueous channels with a physical diameter of ~9 nm (Paine et al., 1975), active transport of large macromolecules through the NPC involves a central gated channel with a functional diameter of up to 26 nm (Feldherr et al., 1984).

A number of specimen preparation methods and electron microscopy (EM) techniques have revealed the major structural components of the NPC (Unwin and Milligan, 1982; Reichelt et al., 1990; Jarnik and Aebi, 1991; Ris, 1991; Goldberg and Allen, 1992; Ris and Malecki, 1993), and 3-D reconstructions have yielded the architecture of the basic framework of the NPC to a resolution of 5 to10 nm (Hinshaw et al., 1992; Akey and Radermacher, 1993). Moreover, the surface topography of the NPC has been visualized recently by atomic force microscopy (AFM) of both dehydrated specimens (Braunstein and Spudich, 1994; Oberleithner et al., 1994) and NEs kept in a physiological buffer environment (Panté and Aebi, 1993; Goldie et al., 1994).

Over the past 3 years, an ever-increasing number of NPC proteins (called nucleoporins) have been identified and characterized. The genes of many of these nucleoporins, including yeast nucleoporins, have been cloned and sequenced. Taking advantage of the yeast genetics, the phenotypes of several nucleoporin mutants have been examined. Furthermore, employing high-resolution immuno-EM techniques, the epitopes of several of these nucleoporins have now been localized within the 3-D architecture of the NPC. Finally, several complexes of nucleoporins have been identified, isolated, and characterized, thereby opening the possibility of reconstituting distinct NPC components *in vitro*. Despite all this progress, there still remain many questions relating to the conformation, molecular composition and functional role(s) of distinct NPC components (reviewed by Panté and Aebi, 1995a,b). Nevertheless, the 3-D localization of an increasing number of nucleoporins, the protein chemical and structural characterization of distinct NPC components and subcomplexes, and the structural/functional analysis of nucleoporin mutations have provided first glimpses into the molecular details of NPC function.

In contrast, less is known about the molecular structure, and signals and factors that control and mediate nucleocytoplasmic trafficking through the NPC. Early studies demonstrated that although mediated nucleocytoplasmic transport of different ligands uses the same NPC machinery, depending on the transport ligand, it appears to occur via different signal pathways. Recently, however, significant progress has been made in identifying the signals mediating the import of nuclear proteins. In fact, it was already found in the mid-1970s that nuclear proteins contain short stretches of amino acids, called nuclear localization sequences (NLSs), that 'ear-mark' them for import into the nucleus (see, e.g., De Robertis et al., 1978). NLSs have now been identified in a large number of proteins (reviewed by Dingwall and Laskey, 1991; Garcia-Bustos et al., 1991; Boulikas, 1993). The next step in the study of the import of nuclear proteins was to identify the cellular factors recognizing NLSs (reviewed by Gerace, 1992). However, it was not until the development of a digitonin-permeabilized cell system to investigate the import of nuclear proteins *in vitro* (Adam et al., 1990) that identification of the NLS receptor and several cytosolic factors mediating the nuclear import of NLS-bearing proteins was finally achieved. Compared

with nuclear import, nuclear export is still at an early stage: only recently have nuclear export signals (NESs) been identified (Fischer et al., 1995; Wen et al., 1995; reviewed by Gerace, 1995).

In the following discussion, we review recent progress made toward the molecular dissection of the 3-D architecture of the NPC. More specifically, after a brief description of the overall morphology of the NPC, its distinct structural components are defined and characterized in some detail. The bulk of the review is devoted to the identification, cloning and sequencing, protein chemical characterization, and 3-D localization of the protein constituents of the NPC. For more detailed information about NPC function, in particular the signals and factors governing nucleocytoplasmic trafficking through the NPC, the reader is referred to a number of recent reviews covering various aspects of this topic (Davis, 1995; Gerace, 1995; Hicks and Raickhel, 1995; Izaurralde and Mattaj, 1995; Melchior and Gerace, 1995; Panté and Aebi, 1995b; 1996; Simos and Hurt, 1995).

II. MORPHOLOGY OF THE NUCLEAR PORE COMPLEX

To date, most of the structural studies of the NPC have been performed using NEs from *Xenopus laevis* oocytes. Oocytes have the advantage that their nucleus can readily be manually isolated, and the NE is easily spread on an EM grid. Moreover, *Xenopus* oocyte NEs yield extensive arrays of densely packed NPCs (see Figure 1) with about 50 to 60 NPCs per square micrometer (Maul, 1977). As illustrated in Figure 2a, when viewed perpendicular to the plane of the NE, negatively stained *Xenopus* oocyte NPCs appear as ring-shaped particles with a diameter of ~125 nm, and they exhibit a

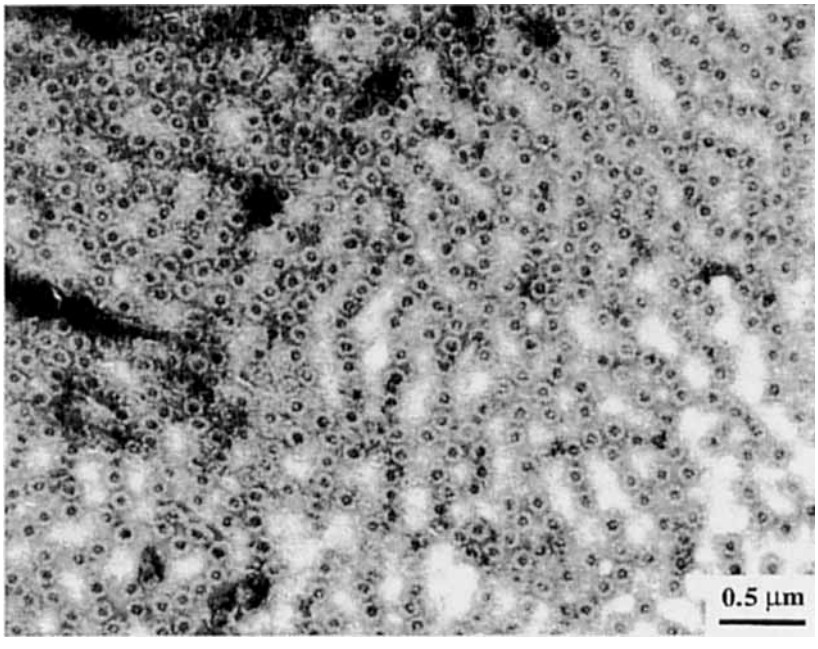

FIGURE 1. *Xenopus* oocyte NEs yield extensive arrays of NPCs. Low magnification overview of an area of a negatively stained spread *Xenopus* oocyte NE. Bar, 0.5 µm.

distinct eightfold rotational symmetry (see also Unwin and Milligan, 1982; Akey, 1989; Reichelt et al., 1990; Jarnik and Aebi, 1991; Hinshaw et al., 1992; Akey and Radermacher, 1993). Not only has the NPC large physical dimensions, but it also yields a gigantic mass: quantitative scanning transmission EM (STEM) has revealed a total mass of 124 MDa (i.e., 124 x 10^6 Da) for the intact, membrane-bound NPC (Reichelt et al., 1990). Assuming that an average nucleoporin has a molecular mass of 100 kDa, about 1200 nucleoporins — most of them probably being present as multiple copies (i.e., 8 or 16 copies because of the 822 symmetry of the basic framework of the NPC; see below) — are required to assemble an NPC. To date, the majority of these nucleoporins are still unidentified (see below).

It appears that the overall morphology of the NPC is largely conserved among different species (Maul, 1977). However, as yet the only moderately detailed structural studies have been performed with *Xenopus* oocyte NPCs, so the conservation of NPC structure across species remains to be established. Toward this goal, the recent development of a procedure to isolate yeast NPCs has opened the possibility of investigating the structure of yeast NPCs in more detail (Rout and Blobel, 1993). Accordingly, whereas the overall morphology of yeast NPCs resembled that of *Xenopus* oocyte NPCs, their diameter was significantly smaller than that of *Xenopus* NPCs. At least in part, this difference may be due to the loss of some NPC components during the biochemical isolation of the yeast NPCs.

While at fist glance the morphology of NPCs from different *Xenopus* oocyte preparations appears to be invariant, a significant degree of structural variability has been depicted among averages of projection images of frozen hydrated NE preparations even within the same micrograph when classified based on multivariable statistical analysis (Akey, 1995). This structural polymorphism has been ascribed to the sampling of distinct functional states during specimen preparation (Akey, 1995), and it may indicate that the NPC has an inherently flexible and hence adaptable structure.

III. STRUCTURAL COMPONENTS OF THE NUCLEAR PORE COMPLEX

As documented in Figures 2b to d, three distinct NPC components are yielded upon solubilization of the nuclear membranes with Triton X-100. (1) 52-MDa 'spoke complexes' (Table 1 and Figure 2b) represent the basic framework of the NPC, which consists of eight multidomain 'spokes' embracing a 'central pore'. Sometimes the central pore is 'plugged' with a particle of variable appearance called the 'central channel complex', 'central plug' or 'transporter'. Based on STEM mass measurements, the mass of the plug-spoke complex is ~ 66 MDa, and that of the unplugged spoke complex ~ 52 MDa (see Table 1; Reichelt et al., 1990). As detailed below, the architecture of the basic framework of the NPC has been determined by 3-D reconstruction of both negatively stained (Hinshaw et al., 1992) and frozen hydrated (Akey and Radermacher, 1993), detergent-treated NPCs. (2) 32-MDa 'cytoplasmic rings' (Table 1 and Figure 2c) appear predominantly positively stained and are also revealed after rolling intact nuclei on an EM grid (Jarnik and Aebi, 1991). (3) 21-MDa 'nuclear rings' (Table 1 and Figure 2d) appear predominantly negatively stained. Consistent with these three distinct NPC components, traditionally (i.e., as depicted in cross-sectional views of embedded/thin-sectioned NEs), the NPC has been described as

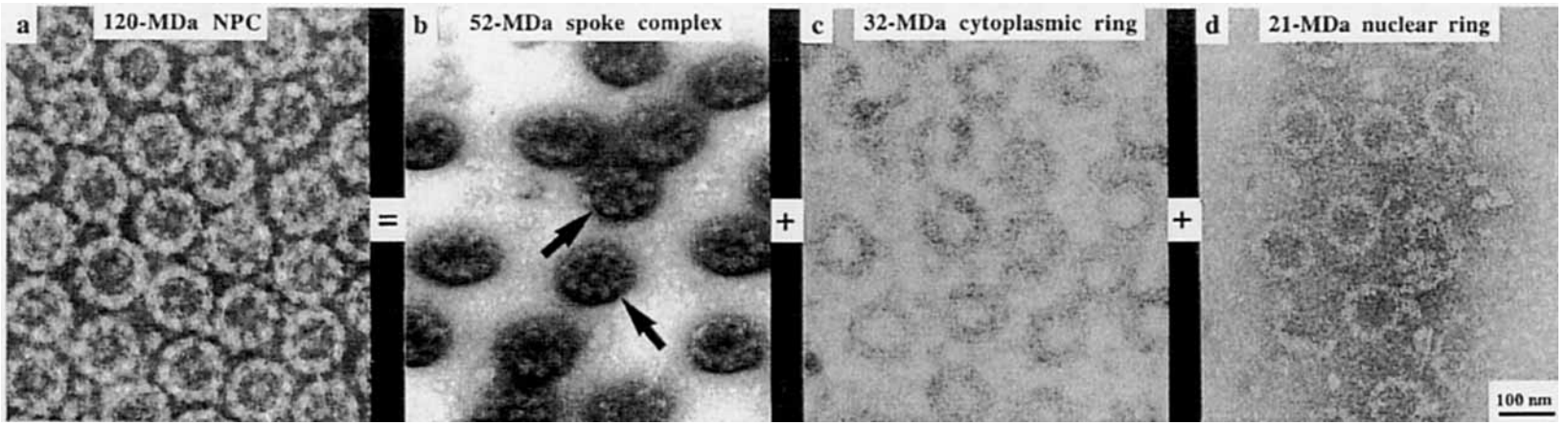

FIGURE 2. The intact NPC is a tripartite supramolecular assembly consisting of a spoke complex sandwiched between a cytoplasmic and a nuclear ring. Electron micrograph of negatively stained intact NPCs (**a**), and of its three distinct components, i.e., the 52-MDa 'spoke complex' or basic framework of the NPC (**b**), the 32-MDa 'cytoplasmic ring' (**c**), and the 21-MDa 'nuclear ring' (**d**), which are yielded after solubilization of the nuclear membrane with 0.1% Triton X-100. **b** represents a 45°-tilt image of negatively stained spoke complexes, some of them containing a central channel complex. The cytoplasmic rings in **c** are also left behind after rolling intact nuclei on a carbon-coated EM grid. Bar, 100 nm (**a-d**).

TABLE 1
Masses of the Intact NPC and its Major Structural Components

	Mass (MDa)[a]
Intact NPC	124.0 ± 11.0
Spoke complex + central channel complex	65.7 ± 8.3
Spoke complex	51.7 ± 5.3
Central channel complex	12.0 ± 1.1
Cytoplasmic ring	32.0 ± 5.5
Nuclear ring	21.1 ± 3.7

[a] Determined by quantitative scanning transmission EM (STEM) and adapted from Reichelt et al. (1990).

a tripartite assembly consisting of the spoke complex sandwiched between a cytoplasmic and a nuclear ring.

Although at first glance the cytoplasmic and nuclear rings have a similar appearance, they are clearly distinct in terms of their mass as determined by quantitative STEM (see Table 1; Reichelt et al., 1990). Accordingly, the cytoplasmic ring is about 30% heavier than the nuclear ring, thus rendering the NPC asymmetric with respect to its cytoplasmic and nuclear periphery. In fact, this difference may also be depicted in projection maps of correlation-averaged NPCs (Jarnik and Aebi, 1991). Moreover, it has also been documented that the cytoplasmic and nuclear periphery of the NPC behave differently in response to limited proteolysis (Goldberg and Allen, 1993). Accordingly, tryptic digestion of NPCs sequentially removes the mass of both rings, however, the nuclear ring is more susceptible to proteolysis than the cytoplasmic ring, indicating that the two rings contain at least in part distinct molecular constituents.

As the 3-D reconstruction of the basic framework of the NPC (Hinshaw et al., 1992; Akey and Radermacher, 1993) yields two tenuous rings at its cytoplasmic and nuclear face (see below and Plate 2a), it has been argued that the cytoplasmic and nuclear rings, as revealed in Figure 2c and d, are integral parts of the basic framework of the NPC. However, the tripartite structure of the NPC depicted by edge-on views of negatively stained (Unwin and Milligan, 1982; Hinshaw et al., 1992) or ice-embedded NPCs (Akey, 1989) and cross-sections of resin-embedded NEs (Jarnik and Aebi, 1991) cannot be solely explained by the basic framework of the NPC. Moreover, in order to arrive at a mass of ~110 MDa (i.e., for the native NPC lacking the central channel complex), the masses of the cytoplasmic and nuclear rings have to be added to that of the basic framework (i.e., 52 MDa + 32 MDa + 21 MDa = 105 MDa; see Table 1 and Reichelt et al., 1990). Hence, in addition to the central channel complex, there must be further components associated with the basic framework of the NPC to account for the ~124-MDa mass of the intact NPC as determined by quantitative STEM (Reichelt et al., 1990).

A. 3-D Architecture of the Basic Framework of the NPC

The 3-D architecture of the basic framework of the NPC (i.e., the spoke complex)

* Plate 2 appears following page 184.

has been determined by 3-D reconstructions of both negatively stained (Hinshaw et al., 1992) and frozen hydrated (Akey and Radermacher, 1993) *Xenopus* oocyte NEs after detergent treatment. These 3-D reconstructions are very similar, with only minor differences. Whereas the map obtained in ice includes an elaborate, barrel-like central channel complex (Akey and Radermacher, 1993), due to its variable appearance in negatively stained preparations, this NPC component has been omitted in the corresponding 3-D map (Hinshaw et al., 1992). However, because the tomographic reconstruction of ice-embedded NPCs contains a relatively large missing cone of information that affects the final 3-D map predominantly at low radii, the central mass within this 3-D map may have been somewhat overestimated despite application of a solvent flattening procedure to the reconstruction (Akey and Radermacher, 1993). In addition, other NPC components (i.e., the nuclear basket, which may have been squashed into the central pore; see below) may contribute significantly to what in ice-embedded NPC images appears as the central channel complex. Moreover, because the abundance and morphology of the central channel complex depend on the isolation and preparation conditions employed (see below), it is necessary to develop procedures that more rigorously control the presence and appearance of the central channel complex residing within the central pore of NPCs before investing too much time and elaborate processing procedures to compute a 3-D map of a highly variable NPC component.

As documented in Plate 2a, the 3-D map of negatively stained, detergent-treated NPCs (Hinshaw et al., 1992) consists of a ring-like framework made of eight multidomain spokes. Each spoke is built of two approximately identical halves (Plate 2b); hence, the entire spoke complex yields 822 symmetry with one half-spoke representing the asymmetric unit. Because this basic framework of the NPC has a mass of 52 MDa (see Table 1 and Reichelt et al., 1990), the mass of one half-spoke is ~3.3 MDa (i.e., on the order of a ribosome). As illustrated in Plate 2b, each spoke is built from two copies each of four distinct morphological domains termed the 'annular' (a), 'column' (c), 'lumenal' (l), and 'ring' (r) domains. The radially outermost domain of each spoke (i.e., the lumenal domain) protrudes into the lumen of the double-membraned NE. It is believed to anchor the spoke complex to the nuclear membranes, and it contains as one of its major constituents the nucleoporin gp210 (see below).

As indicated in Plate 2a, when the inner and outer nuclear membranes are positioned within the 3-D map of negatively stained detergent-treated NPCs, eight ~10-nm-diameter 'peripheral channels' are created between two adjacent spokes and the pore membrane border at a radius of ~40 nm from the NPC center. The 3-D map of ice-embedded, detergent-treated NPCs (Akey and Radermacher, 1993) also yields distinct ~10-nm-diameter channels but, at difference with the negatively stained map (see Plate 2a), these are delineated by two adjacent spokes and the central channel complex at a radius of ~32 nm. As speculated by Hinshaw et al. (1992), these peripheral channels may represent sites for the passive diffusion of ions and small molecules, and they may also facilitate the import of inner nuclear membrane proteins (Soullan and Worman, 1993; reviewed by Wiese and Wilson, 1993).

A 3-D reconstruction of ice-embedded, membrane-associated NPCs has also been determined (Akey and Radermacher, 1993). While at first glance the overall architecture of the NPC yielded in this map seems to be approximately the same as that of the two maps of detergent-released NPCs, closer comparison has revealed significant differ-

ences in the location, orientation, and mutual interactions of the spoke domains. In fact, it has been speculated that this structural variability may have functional significance (Akey and Radermacher, 1993; Akey, 1995). Most likely, these changes are due to the loss of some NPC components, followed by structural rearrangements within the spoke complex upon removing the nuclear membranes. On the other hand, as revealed in micrograph averages (Akey, 1995), membrane-associated NPCs yielded different structures even within a given NE field (i.e., as depicted in an individual micrograph). While it is tempting to speculate that this structural variability may reflect different functional states as they exist within the NE at the time it was isolated and prepared for EM, such an interpretation requires rigorous control of structural and/or functional changes that might possibly be induced in response to the various isolation and preparation steps. As the NPC probably is a rather 'spongy' and thus inherently flexible structure, the observed structural differences may reflect 'more or less systematic deformations' rather than distinct functional states as suggested by Akey (1995). Hence in order to elucidate the 3-D architecture of the native NPC, it is essential to develop isolation/preparation procedures during which the functional and conformational states of the NPC can be rigorously assessed.

B. Peripheral Components of the NPC

As illustrated in Figure 3a, in cross-sections of resin embedded-NEs, the NPC appears interposed between the inner and outer nuclear membrane, where the two membranes are fused to form the 'pore membrane'. In the case of *Xenopus* oocytes, fibrils can be seen protruding from the NPC into both the cytoplasm (see Figure 3a, big arrowheads) and the nucleus (see Figure 3a, small arrowheads). In fact, the presence of these fibrils is also manifested in negatively stained preparations. As documented in Figure 3b and C, *Xenopus* oocyte NEs can be spread on an EM grid so as to expose either their cytoplasmic or their nuclear face. When such spread NE preparations are negatively stained and observed in the EM, the cytoplasmic and nuclear faces of the NPC appear very different. When the NE is adsorbed to the support film with its nuclear face, well-preserved NPCs with a distinct eightfold rotational symmetry are revealed (Figure 3b). In contrast, when the NE is adsorbed to the support film with its cytoplasmic face (i.e., the more frequent occurrence), irregularly stained and poorly preserved NPCs are yielded with *no* clear indication of eightfold rotational symmetry (Figure 3c). Hence, both embedding/thin sectioning and negative staining of isolated NEs demonstrate that the cytoplasmic and nuclear periphery of the NPCs are highly asymmetric.

The morphology and conformation of these peripheral filamentous components of the NPC has been further revealed by two different specimen preparation methods: (1) high-resolution scanning EM (HRSEM) of critical point-dried/metal-sputtered NEs (Ris, 1991; Goldberg and Allen, 1992), and (2) transmission EM of quick-frozen/freeze-dried/rotary metal-shadowed NEs (Jarnik and Aebi, 1991). In Figure 4, the results obtained by the second technique are presented, but HRSEM has revealed similar structures. Accordingly, the cytoplasmic ring is decorated with eight, short kinky filaments (see Figure 4a), whereas the nuclear ring is capped with a basket-like assembly made of eight thin, 50- to 100-nm-long filaments joined distally by a 30- to 50-nm-diameter terminal ring (see Figure 4b).

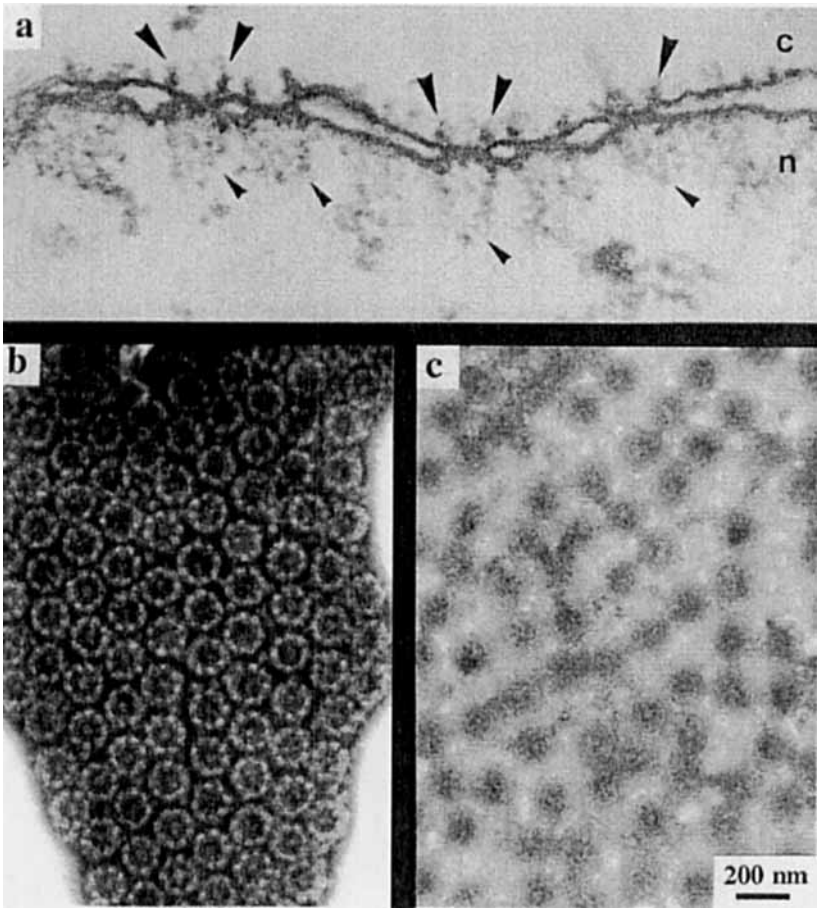

FIGURE 3. The NPC is highly asymmetric with respect to its nuclear and cytoplasmic periphery. (**a**) Cross-section of Epon-embedded nucleus isolated from a *Xenopus* oocyte. In this view, filamentous structures are revealed associated with both the cytoplasmic (large arrowheads) and the nuclear (small arrowheads) face of the NPC. **c** marks the cytoplasmic side of the NE; n, the nuclear side of the NE. (**b**) Cytoplasmic and (**c**) nuclear face of negatively stained intact *Xenopus* oocyte NEs after spreading on an EM grid. Depending on which face of the NE was adsorbed to the EM grid, the NPCs reveal a different morphology. Bar, 200 nm (**a–c**).

As can be best appreciated in stereo pairs, the cytoplasmic filaments do not yield a unique conformation that is an indication of their very flexible structure. This is documented in Figure 4a, where we have marked examples in which (1) the cytoplasmic filaments have bent to the side so as to adhere to filaments of adjacent NPCs (Figure 4a, small arrowheads), (2) they are collapsing onto themselves and thus often appear as 'short cylinders' or 'cigars' (Figure 4a, large arrowheads), or (3) they have bent so as to reach down into the central pore (Figure 4a, small arrows). At this stage it is difficult to determine which of these conformations, if any, represents the native conformation of the cytoplasmic filaments. Nevertheless, it is conceivable that at least some of the conformations depicted in Figure 4a may indeed represent distinct func-

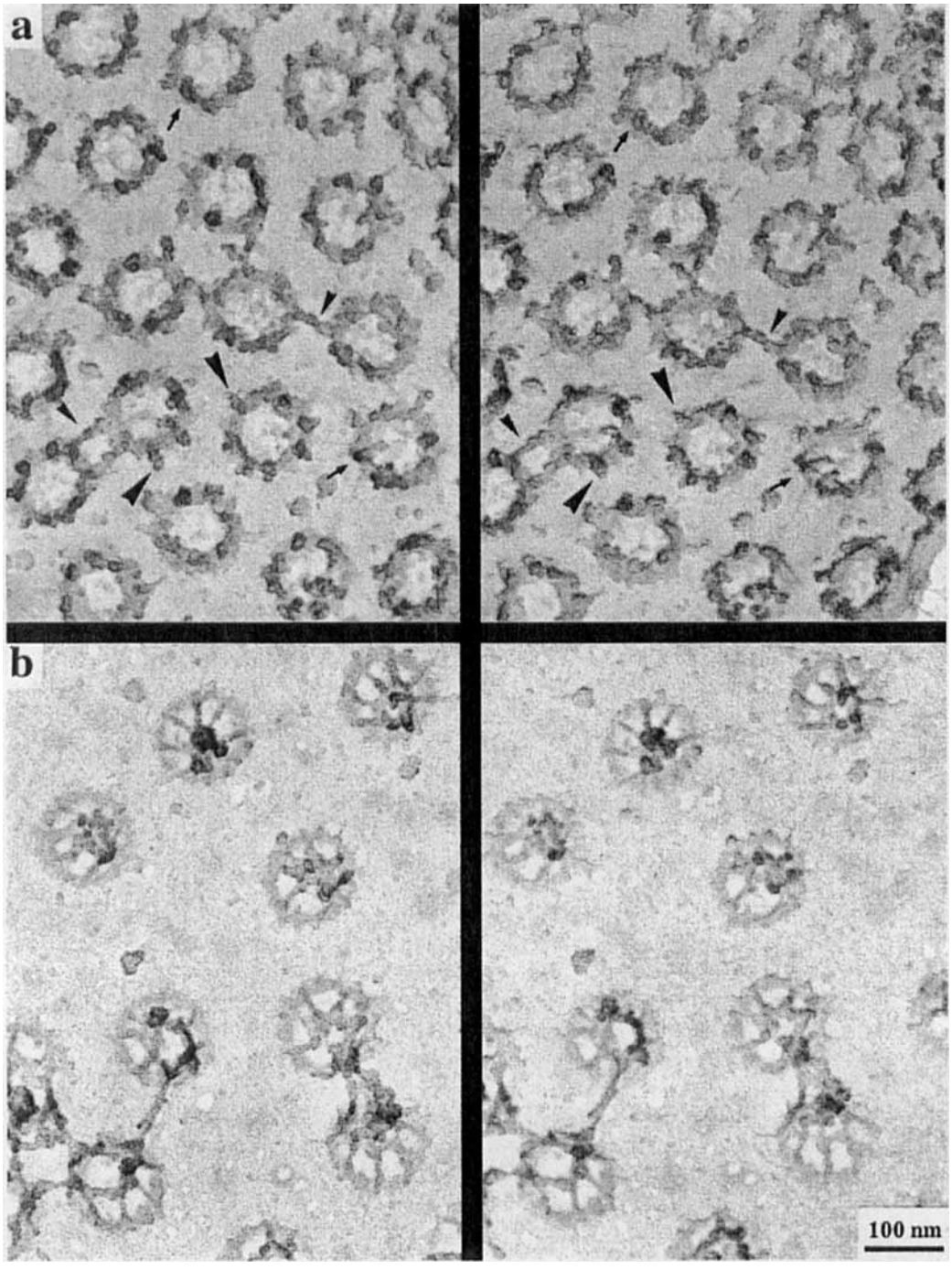

FIGURE 4. Filamentous structures associated with the NPC periphery are revealed by quick freezing/freeze drying/rotary metal shadowing. Stereo pairs of (**a**) the cytoplasmic and (**b**) the nuclear face of quick-frozen/freeze-dried/rotary metal-shadowed intact *Xenopus* oocyte NEs spread on an EM grid. The large arrowheads in **a** point to 'short cylinders' or 'collapsed filaments' protruding from the cytoplasmic rings of the NPCs, whereas the small arrowheads in **a** mark cytoplasmic filaments that have bent to the side and thereby adhered to filaments of adjacent NPCs, thus appearing as 'NPC connecting fibrils'. The small arrows in **a** point to filaments that have bent so as to reach down into the central pore. Bar, 100 nm (**a**, **b**). (Adapted from Jarnik and Aebi, 1991.)

tional states of the cytoplasmic filaments. As to their possible functional role, it has been proposed that the cytoplasmic filaments might be the 'docking sites' for proteins to be imported into the nucleus (Richarson et al., 1988; Gerace, 1992) and hence, by their ability to actively or passively bend, might deliver the docked material to the central channel complex for active translocation.

As documented in Figure 5 (see also Jarnik and Aebi, 1991), the integrity of the nuclear baskets critically depends on the presence of divalent cations during specimen preparation. Whereas well-formed bas-

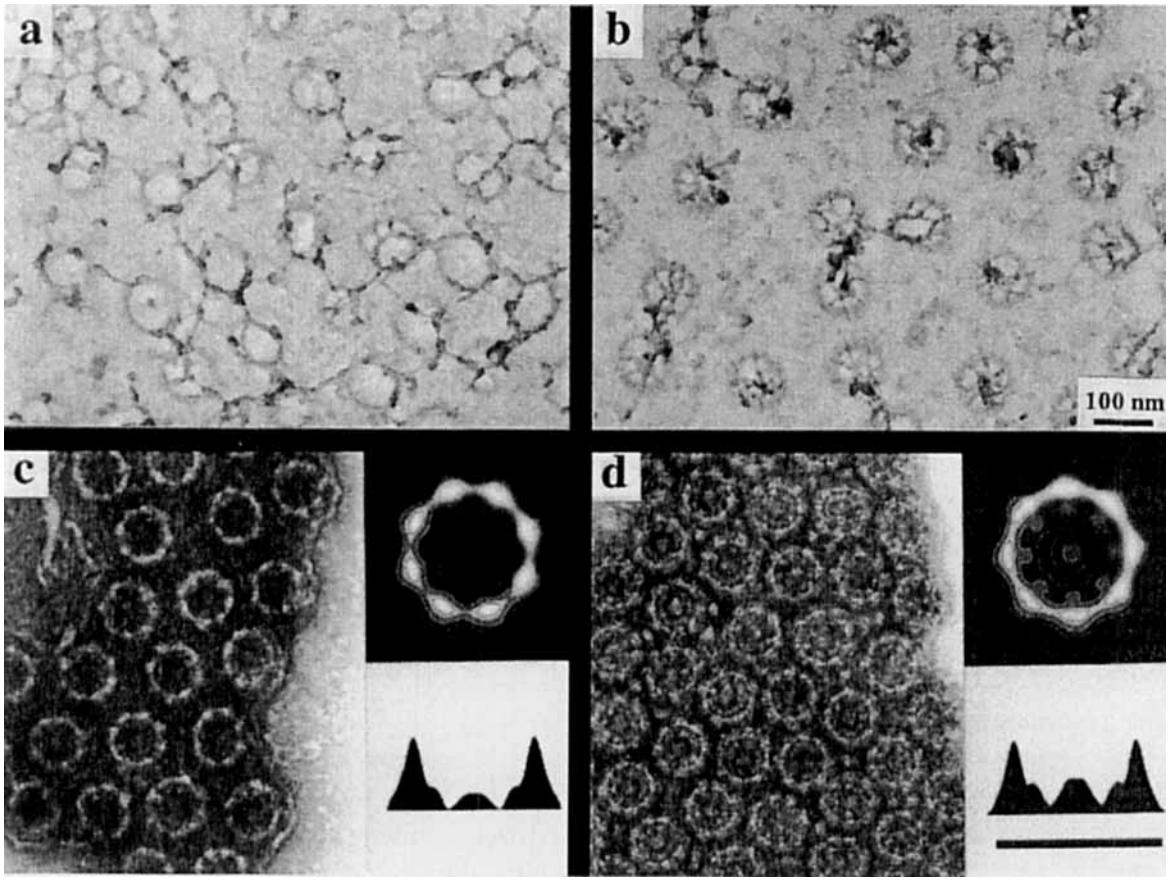

FIGURE 5. The structural integrity of the nuclear baskets depends on divalent cations. (**a**) Nuclear face of a quick-frozen/freeze-dried/rotary metal-shadowed *Xenopus* oocyte spread NE depleted of divalent cations with 2 m*M* EDTA prior to preparation for EM. (**b**) Nuclear face of a *Xenopus* oocyte spread NE after treatment with 2 m*M* EDTA followed by the addition of 0.5 m*M* $MgCl_2$ prior to quick freezing/freeze drying/rotary metal shadowing. Depletion of divalent cations with 2 m*M* EDTA destabilizes the terminal rings and thereby causes disassembly of the nuclear baskets (**a**), whereas the addition of divalent cations after destabilization with EDTA causes reformation of the nuclear baskets (**b**). (**c**, **d**) Electron micrographs, correlation averages (always including 20 eightfold rotationally symmetrized NPCs; upper insets), and radial mass density profiles (lower insets) of negatively stained intact NPCs after treatment with 2 m*M* EDTA (**c**) or in the presence of 0.5 m*M* $MgCl_2$ (**d**). The mass density profile about the center of the NPC (i.e., the appearance of the central plug or channel complex in the micrographs) is significantly attenuated in the presence of 2 m*M* EDTA, a condition that causes disassembly of the nuclear baskets (see **a**). Bar, 100 nm (**a–d**). (Adapted from Jarnik and Aebi, 1991.)

kets are observed if 0.5 m*M* $MgCl_2$ or $CaCl_2$ is present during the isolation and preparation of NEs, chelation of divalent cations by 2 m*M* EDTA or EGTA yields disrupted nuclear baskets (see Figure 5a). Surprisingly, if after destabilization by EDTA or EGTA divalent cations are added back, the nuclear baskets reform (see Figure 5a). Moreover, as depicted in the mass density profile of negatively stained NPCs (Figure 5c and d, lower insets), depletion of divalent cations by EDTA or EGTA yields NPCs with their central mass attenuated (see Figure 5c), compared with control NEs prepared for EM in the presence of millimolar amounts of divalent cations (see Figure 5d). These results indicate that the nuclear basket is a 'dynamic' structure in that it disassembles after removal of divalent cations and reassembles upon their addition (Jarnik and Aebi, 1991). Therefore, the nuclear basket might be directly involved in the active transport of proteins, RNAs or RNP particles trough the NPC.

More recently, the native cytoplasmic and nuclear periphery of the NPC has been visualized by atomic force microscopy (AFM) of spread *Xenopus* oocyte NEs kept in physiological buffer (Panté and Aebi, 1993; Goldie et al., 1994). In agreement with the results revealed with dehydrated specimens (Jarnik and Aebi, 1991; Ris, 1991; Goldberg and Allen, 1992), the corresponding AFM topographs exhibited a high degree of asymmetry between the nuclear and cytoplasmic periphery of the NPC. As documented in Figure 6, by AFM the cytoplasmic face of the NPC appears 'donut-like' (Figure 6a), whereas the nuclear face yields a 'dome-like' appearance (Figure 6b). However, because the resolution in these AFM images is insufficient to resolve individual NPC-associated filaments, the *in vivo* conformation of these cytoplasmic and nuclear filaments has remained uncertain. Nevertheless, this technique has definitely opened the possibility of depicting structural changes of the NPC during the nucleocytoplasmic transport of macromolecules through the NPC. Toward this goal, Bustamante et al. (1995) recently demonstrated by AFM docking of nuclear proteins to the cytoplasmic periphery of the NPC.

In addition to the cytoplasmic filaments and nuclear baskets, HRSEM of critical point-dried/metal-sputtered isolated NEs has revealed in *Triturus cristatus* NEs, but not in *Xenopus* NEs, the existence of a well-ordered fibrous nuclear lattice termed the 'nuclear envelope lattice' or 'NEL' that is connected to the nuclear baskets via their terminal rings (Goldberg and Allen, 1992). The chemical composition and functional significance of this NEL, however, remains to be established. Remnants of such a lattice or filament system connecting adjacent baskets has been observed previously in *Xenopus* oocyte NEs in the form of 'basket connecting filaments' (Jarnik and Aebi, 1991; Ris, 1991).

C. The Central Channel Complex

In projection images of both negatively stained (see, e.g., Figures 2a and 3b) and frozen hydrated intact or detergent-treated NEs, the central pore of the NPC often appears 'plugged' with a ~12 MDa particle (Reichelt et al., 1990) termed the 'central channel complex', 'central plug' or 'transporter'. However, depending on the isolation procedure and/or specimen preparation method employed, this central channel complex yields a highly variable appearance (reviewed by Panté and Aebi, 1994a). The frequent lack of this central structure and its highly polymorphic appearance within a given NPC population has given rise to suggestions that at least in part it may represent particles (i.e., ribonucleoprotein [RNP]

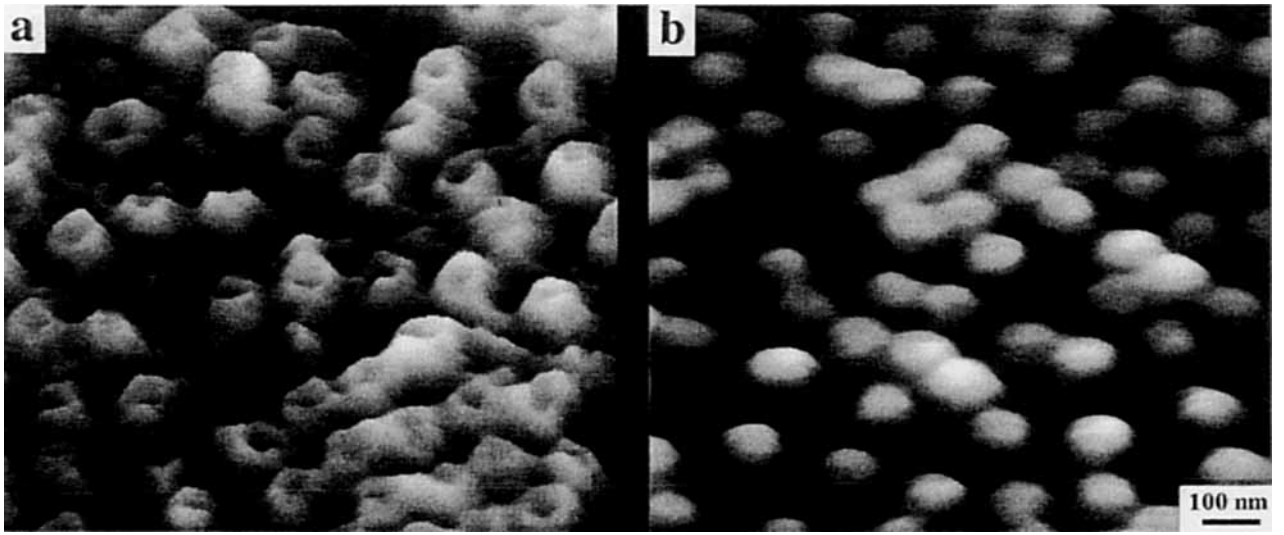

FIGURE 6. Cytoplasmic and nuclear surface topography of NPCs revealed by atomic force microscopy (AFM). Intact *Xenopus* oocyte NEs were spread on carbon-coated glass cover slips, thereby exposing their cytoplasmic (**a**) or nuclear (**b**) face. The spread NEs were kept in their native buffer environment for recording the AFM images. Notice the asymmetric appearance of the cytoplasmic and nuclear faces: the cytoplasmic face of the NPCs appears 'donut-like' (**a**), whereas their nuclear face exhibits a 'dome-like' appearance (**b**). Bar, 100 nm (**a**, **b**).

particles) in transit rather than an integral component of the NPC (e.g., Jarnik and Aebi, 1991; Gerace, 1992). Nevertheless, based on a computer analysis of several thousand NPCs from frozen-hydrated NEs, Akey (1990) has classified the morphology of this central structure into four distinct groups that were related to different transport states of the NPC and hence termed 'closed', 'docked', 'open/in transit', and 'open'. Based on this computer classification, Akey (1990) proposed this central structure to represent the actual 'transporter' and modeled it as a double-iris arrangement that can assume several distinct configurations as it actively transports molecules and particles through the NPC. Because, depending on the isolation and/or specimen preparation of NEs employed, both the abundance and appearance of this central channel complex are highly variable (Unwin and Milligan, 1982; Reichelt et al., 1990; Jarnik and Aebi, 1991), it is far from clear whether the four different morphologies identified by Akey (1990) do indeed represent distinct transport-related states of the central channel complex. Thus, it remains to be established to which extent this central structure does indeed represent an *integral* component of the NPC. Moreover, to establish the functional significance of the four morphologies of this central structure identified by Akey (1990), it is necessary to directly correlate these with corresponding transport assays.

Based on the similarity of its overall size and shape, octagonal symmetry, and mass, it has been proposed that the central channel complex may represent a RNP particle called 'vault' (Kedersha et al., 1991), with the vaults acting as 'transport vehicles' (i. e., carrying nuclear proteins as their cargo). Evidence for vault immunoreactivity at the NPC (Chugani et al., 1993) supports this idea. However, a more specific and comprehensive characterization of the central channel complex in terms of its molecular structure and association with the basic framework of the NPC must be achieved, before it can be identified with any known particle such as vaults. Similarly, any such candidate particles have to be subjected to a more stringent analysis regarding their possible interaction or association with the NPC.

In view of the distinct peripheral structures of the NPC described above (see also Figure 4), it is conceivable that a significant fraction of what in projection appears as the central channel complex in fact represents remnants of the nuclear basket (including the terminal ring) that may have been squashed into the central pore after EM preparation of the NE. In support of this notion, the mass density profile of negatively stained NPCs in the presence of EDTA (see Figure 5c, lower inset), a condition that causes disassembly of the nuclear baskets (see Figure 5a; Jarnik and Aebi, 1991), is significantly attenuated when compared with that of NPCs isolated and prepared in the presence of millimolar amounts of divalent cations (see Figure 5b, lower inset), a condition that stabilizes the nuclear baskets (see Figure 5b; Jarnik and Aebi, 1991). Thus, to more rigorously investigate the nature and 3-D structure of the central channel complex, it is necessary to first develop an isolation/preparation procedure that yields a more reproducible appearance of this NPC component, and at the same time keeps control over the structural integrity of the nuclear baskets. Toward achieving this goal, we have been exploring a wide variety of buffers, incubation conditions, and chemical fixation protocols in term of their possible effects on NPC appearance and morphology. As documented in Figures 7a and b, we found that compared with a control (Figure 7c), ~95% of the NPCs harbor a massive central channel complex when NEs are stabilized with Cu-orthophenanthroline during

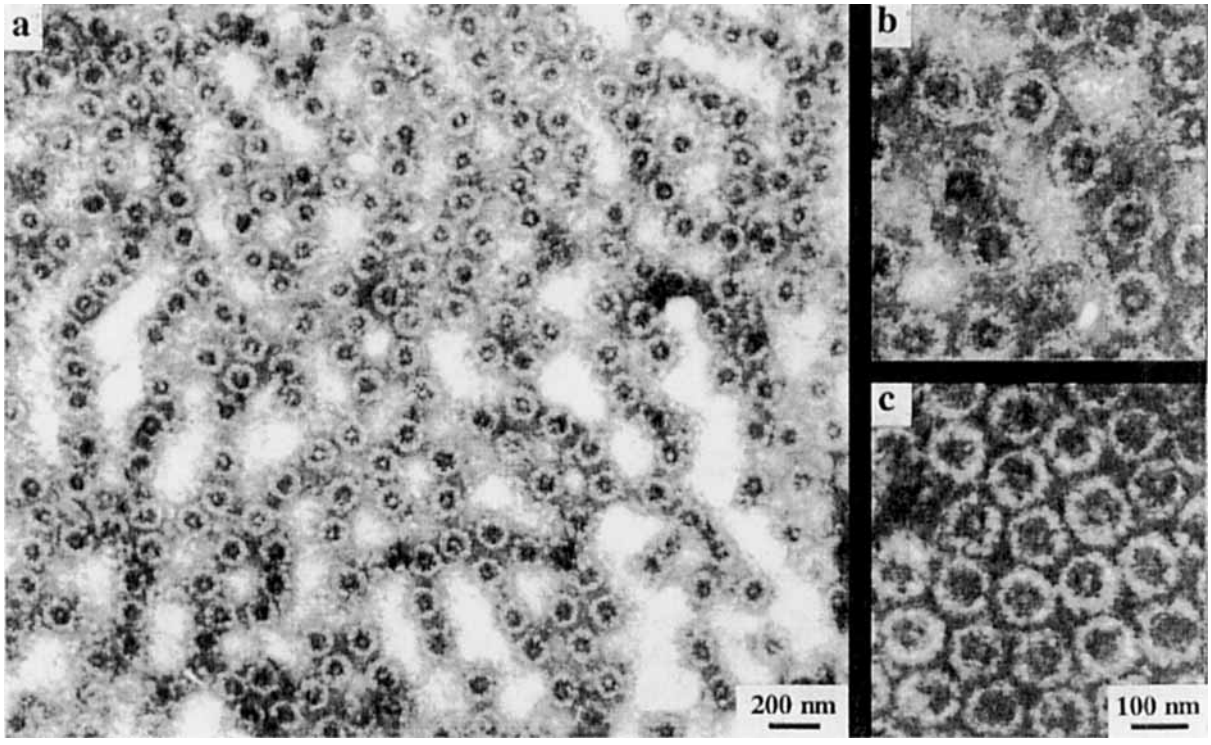

FIGURE 7. Chemical manipulation of isolated NEs changes both the abundance and appearance of the central channel complex of the NPCs. (**a**, **b**) Negatively stained spread *Xenopus* oocyte NEs that have been isolated in the presence of 0.1 m*M* Cu-orthophenanthroline, an oxidizing agent causing intra- and intermolecular S-S bridge formation. (**c**) Control, prepared as in **a**, **b** but in the absence of Cu-orthophenanthroline. About 95% of the NPCs yield a massive central channel complex when they are stabilized with Cu-orthophenanthroline during isolation. Bar, 200 nm (**a**), and 100 nm (**b**, **c**).

isolation, an oxidizing agent catalyzing intra- and intermolecular S-S bridge formation. In contrast, NPCs lacking a central channel complex are yielded if exogenous ATP is added during specimen preparation (Panté and Aebi, unpublished data).

Another possibility is that the central channel complex may not be a permanent component of the NPC in the sense that some of its molecular constituents might be recycled during nucleocytoplasmic transport. For example, some cellular factors mediating nucleocytoplasmic transport might bind to, or associate with, the central channel complex, whereas others, which have previously been bound, might be released together with the transport ligand while being translocated through the central pore. In support of this idea, SRP1, the yeast homolog of importin α (Görlich et al., 1994), a cytosolic factor mediating the import of nuclear proteins, was initially assumed to represent a bona fide constituent of the NPC (Belanger et al., 1994). Moreover, some cellular factors promoting nuclear export have been found both in the nucleus and associated with NPCs, and their primary structure has revealed nucleoporin-like amino acid sequence motifs (Bogerd et al., 1995; Fritz et al., 1995; Stutz et al., 1995). Additional evidence in favor of a more dynamic rather than static molecular composition of the NPC was the finding that the nucleoporin p62 is located at both the nuclear

and cytoplasmic periphery of the central channel complex (see below and Figure 9; Guan et al., 1995), suggesting that this nucleoporin, most likely in complex with other proteins, might be shuttled back and forth between the cytoplasmic and nuclear face of the central channel complex during active translocation of particles through the NPC.

IV. PROTEIN CONSTITUENTS OF THE NPC

Despite recent progress in identifying and characterizing the protein constituents of the NPC (i.e., the nucleoporins), the molecular composition of the NPC is far from being elucidated. Based on its mass of about 120 MDa (Reichelt et al., 1990), with the mass of the asymmetric unit of the basic framework (i.e., one half-spoke) being on the order of 3.3 MDa (see Section III.A. above), it is estimated that the NPC may be built of multiple copies (i.e., 8 or 16, because of the 822 symmetry of the basic framework) of 100 or more different nucleoporins. In addition, it is conceivable that very much like the ribosome, the NPC might also harbor specific RNAs. In recent years an increasing number of nucleoporins from diverse organisms have been identified and characterized, and many of them have been cloned and sequenced (reviewed by Panté and Aebi, 1994b; Rout and Wente, 1994; Bastos et al., 1995). However, the nucleoporins thus far identified represent at most ~15% of the entire NPC mass, and these are predominantly constituents of the cytoplasmic and nuclear periphery rather than of the basic framework of the NPC. Therefore, we have to go a long way to dissect the complete *molecular* architecture of the NPC. Moreover, relatively little is known concerning the native conformation of the nucleoporins thus far identified, their spatial extent within the 3-D structure of the NPC, their mutual interactions to form distinct subcomplexes within the NPC, and their functional roles. One of the major problems with the localization of nucleoporins has been the cross-reactivity of the available antibodies. Using more specific antibodies combined with high resolution immuno-EM protocols, there have been some advances recently to localize epitopes of some of the known nucleoporins within the 3-D architecture of the NPC (see below and Figures 9 to 13). In the following three sections, we summarize what is known of the presently identified nucleoporins from both higher eukaryotes and yeast.

A. VERTEBRATE NUCLEOPORINS

Due to the difficulty in isolating and purifying NPCs from vertebrate species, identification and characterization of vertebrate nucleoporins has been delayed. Nevertheless, a few of these nucleoporins have now been identified using immunological and genetic approaches, and several of them have been molecularly characterized and localized within the 3-D architecture of the NPC. As summarized in Table 2, two major groups of vertebrate nucleoporins have thus far been identified and characterized. These are (1) integral membrane proteins, which are associated with the nuclear membrane and therefore are not extracted upon treatment with alkaline pH or chaotropic agents (Gerace et al., 1982); and (2) peripheral membrane proteins, which are not associated with or anchored in the nuclear membrane. According to a recently proposed nomenclature, the integral membrane proteins of the NPC have been denoted 'POMx' (for pore membrane protein; Hallberg et al.,

TABLE 2
Vertebrate Nucleoporins

Type	Name	Molecular mass[a] (kDa)	Location	Characteristics of primary and predicted secondary structure	Properties and possible functions	Ref.
Integral Membrane Proteins of the NPC	gp210	210	Most of its mass (N domain[b]) resides in the lumen of the NE	21-residue-long transmembrane domain between a 58-residue-long C domain and a 1783-residue-long N domain	Bears N-linked (via Asp) high-mannose oligosacharides Binds ConA Antibodies against lumenal domain inhibit NPC function Possibly anchors the NPC to the pore membrane	Gerace et al., 1982; Wozniak et al., 1989; Greber et al., 1990
	POM121	121	Most of its mass (C domain[c]) resides in the NPC proper	44-residue-long transmembrane domain between a 28-residue-long N domain and a 1127-residue-long C domain C-terminal 1/3 contains repetitive XFXFG motifs	Binds WGA Possibly anchors the NPC to the pore membrane	Hallberg et al., 1993; Söderqvist and Hallberg, 1994
Peripheral membrane proteins of the NPC	p62	62[d]	Cytoplasmic and nuclear periphery near or at the central channel complex	α-Helical coiled-coil C domain N-domain contains repetitive XFXFG motifs	Binds WGA Required for NPC function Exists as a complex with p58, p54, and p45 (i.e., the p62 complex)	Starr et al., 1990; Carmo-Fonseca et al., 1991; Cordes et al., 1991; Finlay et al., 1991; Kita et al., 1993; Buss and Stewart, 1995; Guan et al., 1995
O-linked glycoproteins	NUP98/p97	98	Nucleus and nuclear periphery of the NPC	N-terminal 1/2 contains repetitive GLFG, FXFG, and FGG motifs	Highly homologous to members of the yeast GLFG family (see Table 3) Not essential for NPC function Required for nuclear growth and replication	Powers et al., 1995; Radu et al., 1995a
	NUP153	153	Near or at the terminal ring of the nuclear basket	C-terminal 2/3 contains repetitive XFXFG motifs Four zinc finger motifs	Binds WGA Exists as a ≥1 MDa homo-oligomer The zinc finger motifs bind DNA *in vitro*	Sukegawa and Blobel, 1993; McMorrow et al., 1994; Cordes et al., 1993; Panté et al., 1994
	CAN/NUP214/p250	214	Cytoplasmic filaments	Contains repetitive XFXFG, SVFG, FGQ, and FGG motifs Leucine zipper motif	Binds WGA Exists as a complex with p75 Putative oncogene product associated with myeloid leukemogenesis	Von Lindern et al., 1992; Kraemer et al., 1994; Panté et. al., 1994

TABLE 2 (continued)
Vertebrate Nucleoporins

Type	Name	Molecular mass[a] (kDa)	Location	Characteristics of primary and predicted secondary structure	Properties and possible functions	Ref.
	RanBP2/NUP358	358	*Cytoplasmic* filaments	*Contains repetitive XFXFG* and FG motifs Eight zinc finger motifs Four Ran-binding domains Leucine zipper motif C terminus homologous to cyclophilin	Binds WGA The zinc finger motifs bind DNA *in vitro* Binds Ran/TC4-GTP Anti-RanBP2 antibodies inhibit NPC functions	Melchior et al., 1995; Wilken et al., 1995; Wu et al., 1995; Yokoyama et al., 1995
Non-O-linked proteins	NUP107	107	Unknown	Leucine zipper motif	Does not bind WGA	Radu et al., 1994
	NUP155	155	Unknown	No repetitive motifs	Does not bind WGA	Radu et al., 1993
	Tpr/p265	265	Cytoplasmic filaments	~1600-residue-long α-helical coiled-coil region Acidic C domain	Susceptible to proteolysis with a major proteolytic product of ~180 kDa	Byrd et al., 1994

a Calculated from the amino acid sequence.
b NH_2-terminal domain
c COOH-terminal domain
d Depending on species.

1993), and the peripheral membrane proteins of the NPC 'NUPx' (for nucleoporin; Wente et al., 1992), where x indicates the predicted molecular mass in kilodaltons based on their amino acid sequence. For the few nucleoporins identified before the introduction of this nomenclature (e.g., gp210, p62), we use the original name.

1. Integral Membrane Proteins of the NPC

a. gp210

The first NPC protein to be identified and isolated from both rat and *Drosophila* NE preparations (Gerace et al., 1982; Filson et al., 1985) was gp210, a glycoprotein bearing N-linked high-mannose oligosaccharides, thus binding the lectin concanavalin A (ConA) (Gerace et al., 1982; Wozniak et al., 1989; Greber et al., 1990). As illustrated schematically in Plate 3, cDNA sequencing and topological studies have indicated that gp210 consists of a large (95% of its total mass) NH_2-terminal domain residing in the NE lumen, a single, 21-residue-long transmembrane segment, and a short, 58-residue-long COOH-terminal domain (Wozniak et al., 1989; Greber et al., 1990). The latter is associated with the NPC proper (Greber et al., 1990), whereas the transmembrane segment is the determinant for sorting gp210 to the membrane domain of the NPC (Wozniak and Blobel, 1992). Based on this topology, there have been speculations that the large lumenal domain of gp210 forms part of the 'knobs' or 'lumenal subunits' that have been shown to extend from the spokes radially into the lumen of the NE (Jarnik and Aebi, 1991; Hinshaw et al., 1992; Akey and Radermacher, 1993). Interestingly, antibodies against the lumenal domain of gp210 inhibit both passive diffusion of small molecules and mediated nuclear import of NLS-bearing proteins (Greber and Gerace, 1992). The suggested role of gp210 is to anchor the NPC in the pore membrane and to exert a topogenic role in membrane folding during nuclear pore formation (Greber et al., 1990; Jarnik and Aebi, 1991; Gerace, 1992; Hinshaw et al., 1992).

b. POM121

A second integral membrane protein of the NPC, POM121, has been identified in rat NEs (Hallberg et al., 1993). Similar to some of the peripheral membrane glycoproteins of the NPC (see below and Table 2), POM121 binds wheat germ agglutinin (WGA), a lectin that recognizes O-linked *N*-acetylglucosamine (GlcNac) residues. As illustrated in Plate 3, the primary structure of POM121 as deduced from its cDNA sequence revealed that this nucleoporin consists of a 44-residue-long transmembrane segment sandwiched between a short 28-residue-long NH_2-terminal domain and a long COOH-terminal domain. The latter contains the repetitive pentapeptide motif XFXFG (where X indicates any amino acid) that is also found in the members of the O-linked NPC glycoprotein family as well as some yeast nucleoporins (see below and Tables 2 and 3).

The membrane topology of POM121 has been elucidated recently by combining immunofluorescence microscopy with differential detergent permeabilization (Söderqvist and Hallberg, 1994). Accordingly, antibodies against the COOH-terminal domain of POM121 label cells treated with digitonin (a condition that permeabilizes only the plasma membrane; Adam et al., 1990) as well as cells treated with digitonin

* Plate 3 appears following page 184.

TABLE 3
Yeast Nucleoporins

Type	Name	Molecular mass[a] (kDa)	Characteristics of primary and predicted secondary structure	Essential for cell growth?	Properties and mutation defects	Ref.
XFXFG family	NSP1	87	α-Helical coiled-coil C domain[b] Central region contains repetitive XFXFG motifs	Yes	C domain essential for function Forms a complex with NUP49 NUP57, and NIC96 NSP1 mutations cause import defects and decreases the number of NPC	Hurt, 1988; Nehrbass et al., 1990; Mutvei et al., 1992; Grandi et al., 1993
	NUP1	114	Central region contains repetitive XFXFG motifs	Yes	Interacts with SRP1 NUP1 mutations cause both import and export defects and changes in NE morphology	Davis and Fink, 1990; Belanger et al., 1994
	NUP2	95	Central region contains repetitive XFXFG motifs	No	Interacts with SRP1 Binds Ran/TC4	Loeb et al., 1993; Belanger et al., 1994 Dingwall et al., 1995
	NUP159/RAT7	159	α-helical coiled-coil C domain Central region contains repetitive XFXFG and FG motifs	Yes	NUP159/RAT7 mutations cause export defects and NPC clustering	Gorsch et al., 1995; Kraemer et al., 1995
GLFG family	NUP49	49	α-Helical coiled-coil C domain Repetitive GLFG motifs within N domain[c]	Yes	Forms a complex with NSP1, NUP57, and NIC96	Wente et al., 1992; Wimmer et al., 1992
	NUP57	57	α-Helical coiled-coil C domain N domain contains repetitive GLFG motifs	Yes	Forms a complex with NSP1, NUP49, and NIC96	Grandi et al., 1995b
	NUP100	100	N domain contains repetitive GLFG motifs RNA binding motifs	No	Highly homologous to NUP116	Wente et al., 1992; Fabre et al., 1994.
	NUP116	116	N domain contains repetitive GLFG motifs RNA binding motifs	No	Binds RNA *in vitro* Deletions in the *nup116* gene yield sealed NPCs	Wente et al., 1992; Wimmer et al., 1992; Wente and Blobel, 1993; Fabre et al., 1994
	NUP145	145	N domain contains repetitive GLFG motifs RNA binding motifs	Yes	Binds RNA *in vitro* Deletions/disruptions in the *nup145* gene yield clusters of sealed NPCs	Fabre et al., 1994; Wente and Blobel, 1994

Nucleoporins with nonrepetitive sequence motifs	NIC96	96	α-Helical coiled-coil N domain	Yes	Forms a complex with NSP1, NUP49 and NUP57 Depletion of NIC96 causes import defects	Grandi et al., 1993
	NUP82	82	α-Helical coiled-coil C domain	Yes	Depletion of NUP82 causes export defects	Grandi et al., 1995a; Hurwitz and Blobel, 1995
	NUP120/RAT2	120	Leucine zipper motif Similar to NUP133/RAT3	No	Deletions in the *nup120/rat2* gene cause export defects, NPC clustering, and nucleolar fragmentation	Aitchison et al., 1995b; Heath et al., 1995
	NUP133/RAT3	133	Two 16- to 18-residue-long hydrophobic stretches	No	Disruptions in the *nup133/rat3* gene cause export defects and NPC clustering	Doye et al., 1994; Li et al., 1995; Pemberton et al., 1995
	NUP157	157	Similar to NUP170 and vertebrate NUP155	No	Abundant yeast nucleoporin	Aitchison et al., 1995a
	NUP170	170	Similar to NUP157 and vertebrate NUP155	No	Abundant yeast nucleoporin Depletion of NUP170 causes morphological abnormalities in the NE	Aitchison et al., 1995a
	POM152	152	20-residue-long transmembrane domain between a 175-residue-long N domain and a 1142-residue-long C domain	No	Integral membrane protein Most of its mass (C domain) resides in the lumen of the NE Binds ConA	Wozniak et al., 1994

[a] Calculated from the amino acid sequence.
[b] COOH-terminal domain.
[c] NH_2–terminal domain.

followed by Triton X-100 (a condition that permeabilizes the nuclear membranes). In contrast, antibodies against the short NH_2-terminal domain label only Triton X-100-permeabilized cells. Therefore, it was concluded that in contrast to gp210, the small, 28-residue-long NH_2-terminal domain of POM121 resides in the lumen of the NE, whereas the large COOH-terminal domain, containing more than 90% of the mass of POM121, resides within the NPC proper (Plate 3). Very much like gp210, POM121 has been proposed to function as a membrane anchor of the NPC (Hallberg et al., 1993).

2. Peripheral Membrane Proteins of the NPC

a. The O-Linked NPC Glycoprotein Family

A family of at least eight nucleoporins that are modified at up to 10 to 20 sites with O-linked GlcNac, and therefore bind WGA, was originally identified in rat liver NEs (Snow et al., 1987; Holt et al., 1987; Davis and Blobel, 1987). These nucleoporins, estimated by SDS-PAGE, have molecular masses ranging between 45 and 210 kDa, and they are present in roughly 1 to 10 copies per NPC (Holt et al., 1987). Because both WGA (Finlay et al., 1987; Dabauvalle et al., 1988b) and monoclonal antibodies to these proteins (Dabauvalle et al., 1988a; Featherstone et al., 1988) inhibit the import of nuclear proteins, it has been speculated that some of these O-linked glycoproteins might be directly involved in mediated nuclear import. As summarized in Plate 3 and Table 2, five members of the O-linked glycoprotein family have been cloned and sequenced. As a hallmark, their deduced amino acid sequences reveal the presence of distinct repetitive sequence motifs: four of these (p62, NUP153, CAN/NUP214/p250, and RanBP2/NUP358) contain multiple copies (up to 37 in NUP153) of the pentapeptide repeat XFXFG (where X indicates any amino acid), while NUP98/p97 contains multiple copies of the tetrapeptide repeat GLFG. These repetitive sequence motifs are now considered to be a diagnostic feature for nucleoporins, and it has been speculated that they function as docking sites for NLS-bearing proteins (Radu et al., 1995b). Following is a description of some of the properties of these five nucleoporins.

i. p62 and the p62 Complex

p62 is the most abundant member of the O-linked NPC glycoprotein family. Its gene has been cloned and sequenced from *Xenopus*, mouse, rat, and human (Starr et al., 1990; Carmo-Fonseca, et al., 1991; Cordes et al., 1991). Analysis of the primary sequences of the various p62 homologs has indicated that the p62 molecule is constructed from two distinct halves separated by a stretch rich in hydroxylated residues (Plate 3). The NH_2-terminal half (residues 1 to 324 in rat p62) contains 15 to 25 copies of the degenerate pentapeptide motif XFXFG, whereas the COOH-terminal half (residues 325 to 523 in rat p62) reveals heptad repeats that are predicted to form four α-helical coiled-coil segments, each being ~30 to 40 residues long (Starr et al., 1990). In agreement with these structural predictions, recombinant p62 produced in *E. coli* appears as a ~35-nm-long rod-shaped molecule with a slight protuberance at the NH_2-terminal end in the EM after glycerol spraying/rotary metal shadowing (Buss et al., 1994).

p62 can be dissociated from the NPC as a large supramolecular complex, the 'p62 complex', that is required for the formation of functional NPCs (Finlay et al., 1991) and

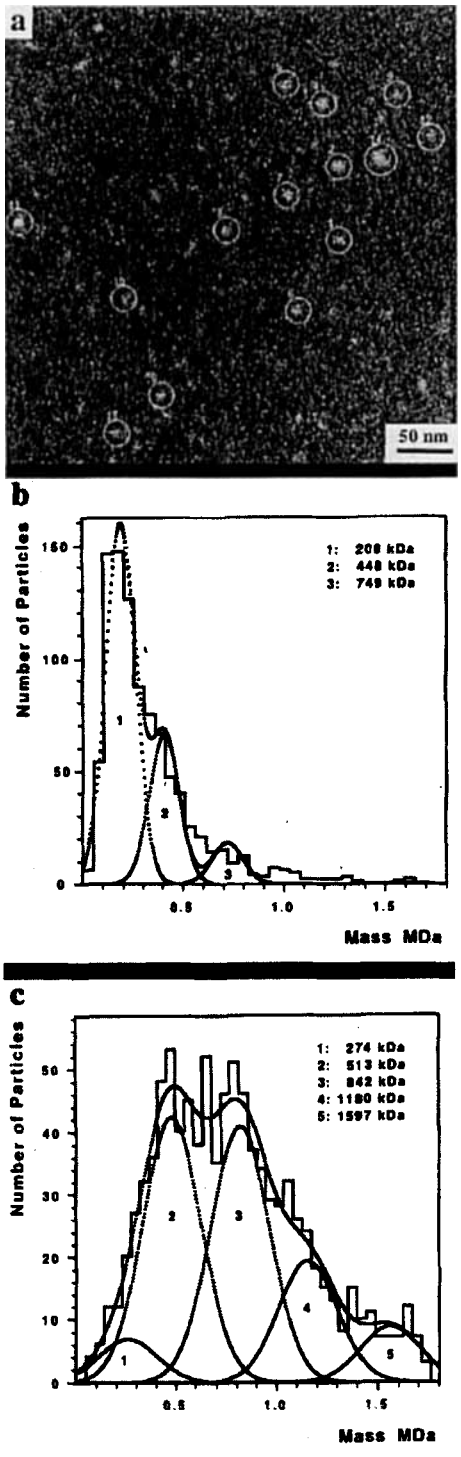

FIGURE 8. The ~220-kDa p62 complex is able to self-associate into higher-order oligomers that appear as donut-like particles of variable size in the EM. (**a**) Low-dose scanning transmission EM (STEM) micrograph of unstained/freeze-dried p62 particles. Examples of particles used for mass determination have been circled. Bar, 50 nm. (**b** and **c**) Mass histograms from native p62 particles as shown in **b**, and from p62 particles that have been stabilized during isolation with 0.1 m*M* Cu-orthophenantroline (**c**). Images were recorded using doses of 314 ± 22 e/nm^2. Each histogram together with its corresponding Gaussian fits displays the mass data measured from 930 p62 particles (not corrected for beam-induced mass loss).

which interacts with NTF2, a cytosolic transport factor (Paschal and Gerace, 1995). The p62 complex was first identified in *Xenopus* oocyte NEs (Dabauvalle et al., 1990), and it has been isolated recently and characterized at the molecular level from rat liver NEs by a number of investigators (Finlay et al., 1991; Kita et al., 1993; Buss and Stewart, 1995; Guan et al., 1995). Accordingly, it consists of p62 interacting with three other nucleoporins of apparent molecular masses of 58 (p58), 54 (p54), and 45 (p45) kDa. The latter is only revealed when extreme care is taken to avoid proteolysis during isolation (Guan et al., 1995). The molecular mass and subunit stoichiometry of the p62 complex have been a matter of controversy. However, the recent development of a modified procedure to isolate the p62 complex has revealed a mass of ~234 kDa, both calculated from its hydrodynamic properties and measured directly by quantitative STEM (Guan et al., 1995). When examined in the EM after negative staining or glycerol spraying/rotary metal shadowing, the p62 complex, when isolated by the procedure developed by Guan et al. (1995), appears as a donut-like particle with an apparent diameter of ~15 nm. STEM mass analysis of these particles (Figure 8a) yields the mass histogram displayed in Figure 8b indicating the presence of a mixture of at least three components with relative masses of 206 kDa (69% of the particles), 448 kDa (26% of the particles), and 749 kDa (5% of the particles). These data suggest that the p62 complex consists of a ~220-kDa particle containing one copy each of the four constituent nucleoporins (i.e., p62:p58:p54:p45= 1:1:1:1). This, 'minimal' p62 complex has a tendency to self-associate into higher-order oligomers that appear as approximately round particles of variable size in the EM (see Figure 8a). These self-aggregation properties of the p62 complex are further documented by chemical cross-linking. As illustrated in the mass histogram shown in Figure 8c, stabilization of the p62 complex during isolation with Cu-orthophenanthroline, an oxidizing agent causing intra- and intermolecular S-S bridge formation, decreased the number of monomeric p62 particles (from 69 to 5%) and shifted the histogram to higher-molecular-weight species. These findings indicate that the minimal, ~230 kDa p62 complex is able to self-associate into higher-order oligomers. It remains to be established, however, which oligomer form(s) are actually present in native NPCs.

Due to the relatively strong cross-reactivity of some of the antibodies against nucleoporins, until recently the localization of p62 within the 3-D structure of the NPC has remained ambiguous (Cordes et al., 1991; Panté and Aebi, 1993). Using a polyclonal mouse anti-p62 antibody, Cordes et al. (1991) located p62 to both the cytoplasmic and nuclear periphery of mouse liver NEs but only to the nuclear face of *Xenopus* oocyte NEs. Another reason for this ambiguity has been the use of EM preparations by which distinct NPC components are poorly preserved and/or not well resolved. To try to resolve this issue, we have used a monoclonal antibody, RL31, that reacts exclusively and specifically with rat p62 (Guan et al., 1995). As illustrated in Figure 9, this antibody labels both the cytoplasmic and nuclear periphery near or at the central channel complex of rat liver NPCs. This localization of p62 is consistent with the presumed involvement of the p62 complex in the import of nuclear proteins (Finlay et al., 1991) or export of RNAs (Dargemont et al., 1995), and it may indicate that the p62 complex associates with material that is actively translocated through the NPC. The observation that the p62 complex is found at both the cytoplasmic and nuclear periphery near or at the central channel complex (Figure 9) suggests that it might be shuttled

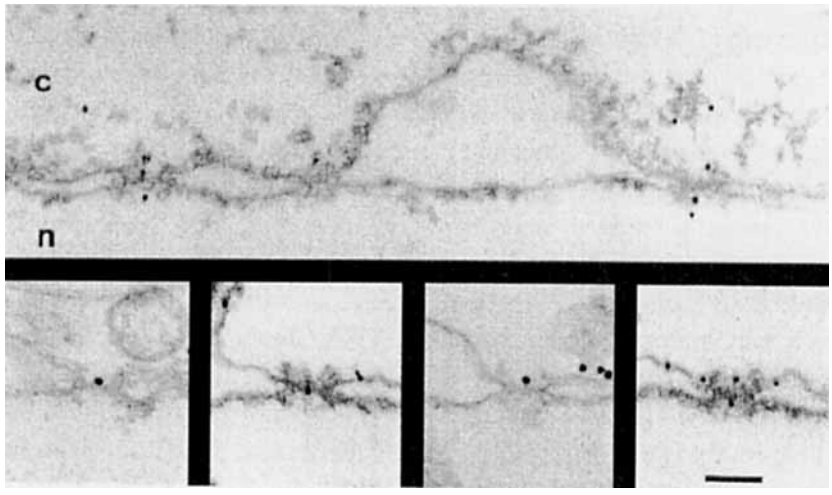

FIGURE 9. p62 is found at both the cytoplasmic and nuclear periphery near or at the central channel complex. Isolated rat liver NEs were incubated with the monoclonal antibody RL31, which reacts exclusively and specifically with rat p62 (Guan et al., 1995), directly conjugated to 8-nm colloidal gold, and prepared for EM by Epon embedding and thin sectioning. Shown is a view along a cross-sectioned NE stretch, together with a gallery of selected examples of gold-labeled NPC cross-sections. These cross-sections reveal that RL31 predominantly labels both the cytoplasmic and nuclear periphery near or at the central channel complex of rat liver NPCs. **c,** the cytoplasmic side of the NE; n, the nuclear side of the NE. Bar, 100 nm.

back and forth between the cytoplasmic and nuclear face of the central channel complex.

ii. NUP153

NUP153 was the second member of the O-linked NPC glycoprotein family whose primary structure was elucidated for both the rat (Sukegawa and Blobel, 1993) and human isoform (McMorrow et al., 1994). Accordingly, the COOH-terminal domain of NUP153 contains up to 37 copies of the degenerate pentapeptide motif XFXFG (Plate 3). In addition, the primary sequence of NUP153 harbors four zinc finger motifs, each containing two pairs of cysteine residues (Cys_2-Cys_2) (Sukegawa and Blobel, 1993; McMorrow, et al., 1994). Because this type of zinc finger motif is found in DNA-binding proteins (reviewed by Coleman, 1992), a fragment of NUP153 containing these four motifs was expressed in *E. coli* and demonstrated to bind DNA in a zinc-dependent manner (Sukegawa and Blobel, 1993). This result has given rise to speculations about a possible role of NUP153 in gating transcribable genes to the NPC, thus facilitating export of the transcribed RNA (Sukegawa and Blobel, 1993). However, it remains to be demonstrated whether NUP153 binds DNA *in vivo*.

Using a polyclonal antibody raised against a fusion protein expressed from a NUP153 cDNA construct, NUP153 has been unequivocally localized to the nuclear periphery of the NPC (Sukegawa and Blobel, 1993). However, in this labeling study NUP153 could not be identified with a dis-

tinct NPC component(s). More specific localization of NUP153 has been achieved independently by two groups. Cordes et al. (1993) localized this protein to intranuclear NPC-attached filaments that, among other structures, may represent nuclear baskets that have been disrupted during sample preparation. Panté et al. (1994) have identified NUP153 as a constituent of the nuclear basket, with at least one of its epitopes residing near or at the terminal ring (Figure 10a). This localization for NUP153 together with its four zinc finger motifs is consistent with the stabilizing effect of Zn^{2+} on the nuclear baskets (Figure 5; Jarnik and Aebi, 1991): in the presence of 0.5 m*M* $ZnCl_2$, well-formed baskets are observed, whereas when divalent cations are chelated by 2 m*M* EDTA or EGTA, the nuclear baskets become destabilized and dissociate. Surprisingly, if after destabilization by EDTA or EGTA divalent cations are added back, the nuclear baskets reform, most efficiently with Zn^{2+} (Jarnik and Aebi, 1991). These findings indicate that the zinc finger motifs of NUP153, in addition to binding DNA or RNA (Sukegawa and Blobel, 1993), may be involved in maintaining the structural integrity of the nuclear baskets, and thereby directly participating in the active transport of proteins, RNAs, or RNP particles through the NPC.

NUP153 can be dissociated from the NPC as a homooligomer with a molecular mass of ≥1 MDa, most likely representing an octamer. As at least part of NUP153 resides within the terminal ring of the nuclear baskets (Figure 10a; Panté et al., 1994), it is conceivable that an octameric NUP153 complex constitutes at least in part a scaffold of the eightfold symmetric terminal ring (Figure 10b).

iii. CAN/NUP214/p250

CAN was originally identified as the product of a putative oncogene (*can*) involved in chromosomal rearrangements associated with human leukemia (Von Lindern et al., 1992). By analyzing partial amino acid sequences of the ~210 kDa O-linked NPC glycoprotein originally identified by Snow et al. (1987), Kraemer et al. (1994) have demonstrated recently that this nucleoporin is the rat homolog of human CAN. Because based on its cDNA-deduced primary structure CAN has a calculated molecular mass of 214 kDa, this nucleoporin was termed NUP214. Using a monoclonal antibody called QE5, Panté et al. (1994) identified a ~250-kDa NPC glycoprotein (termed p250) in extracts of BHK cells. On immunoblots, p250 is recognized by both RL1, a monoclonal antibody that recognizes at least eight O-linked NPC glycoproteins of rat liver NEs (Snow et al., 1987), and antibodies raised against the cloned NH_2-, and COOH-terminal domains of human CAN (B. Burke and R. Bastos, personal communication). Therefore, p250 corresponds to the O-linked glycoprotein CAN/NUP214.

Consistent with being a nucleoporin, the amino acid sequence of CAN reveals a number of degenerate pentapeptide motifs (XFXFG) located predominantly within the COOH-terminal half of the molecule (Plate 3). In addition, CAN contains multiple copies of the tripeptide motif FGQ that is also present in the two yeast nucleoporins, NUP100 and NUP/NSP116 (see below; Wente et al., 1992; Wimmer et al., 1992). Another distinct feature of CAN's primary sequence is the presence of a leucine zipper motif that may act as a protein-protein dimerization domain (Von Lindern et al., 1992).

In an attempt to *in situ* localize CAN/NUP214/p250, this nucleoporin has been expressed as a fusion protein against which a polyclonal antibody was raised (Kraemer et al., 1994). Whereas this affinity-purified antibody clearly labeled the cytoplasmic periphery of the NPC (Kraemer et al., 1994),

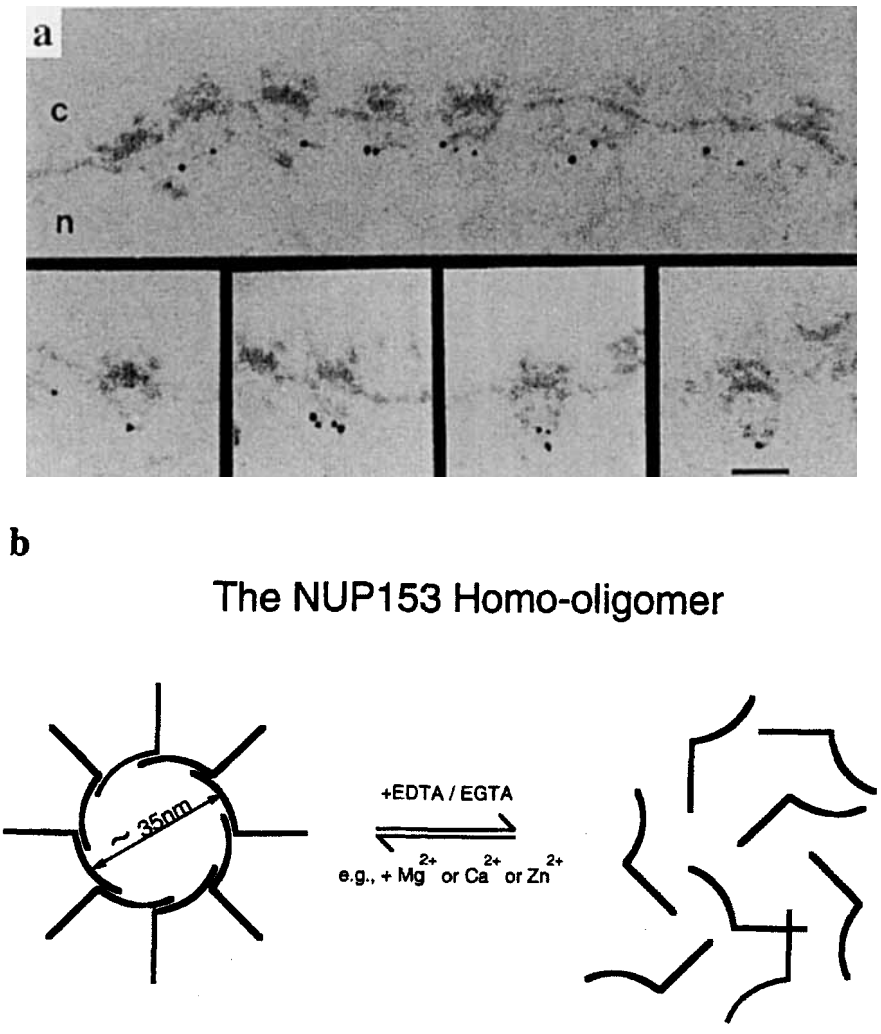

FIGURE 10. NUP153 is a constituent near or at the terminal ring of the nuclear basket of the NPC. (**a**) Triton X-100-treated *Xenopus* oocyte nuclei were labeled with an anti-peptide antibody directed against human NUP153 (Panté et al., 1994) directly conjugated to 8-nm colloidal gold, and prepared for EM by Epon embedding and thin sectioning. Shown is a view along a cross-sectioned NE stretch, together with a gallery of selected examples of gold-labeled NPC cross-sections. These cross-sections document that the anti-NUP153 anti-peptide antibody exclusively labels the terminal ring of the nuclear baskets. c, the cytoplasmic side of the NE; n, the nuclear side of the NE. Bar, 100 nm. (**b**) Schematic diagram of the possible formation of a NUP153 homooligomer representing a distinct component (i.e., the terminal ring) of the nuclear baskets (see Panté et al., 1994).

this labeling was not specific enough to identify CAN/NUP214 with a distinct NPC component(s). As illustrated in Figure 11, using the monoclonal QE5 antibody and a polyclonal antibody raised against gel-purified rat liver p250, Panté et al. (1994) more specifically located this protein to the cytoplasmic filaments of the NPC.

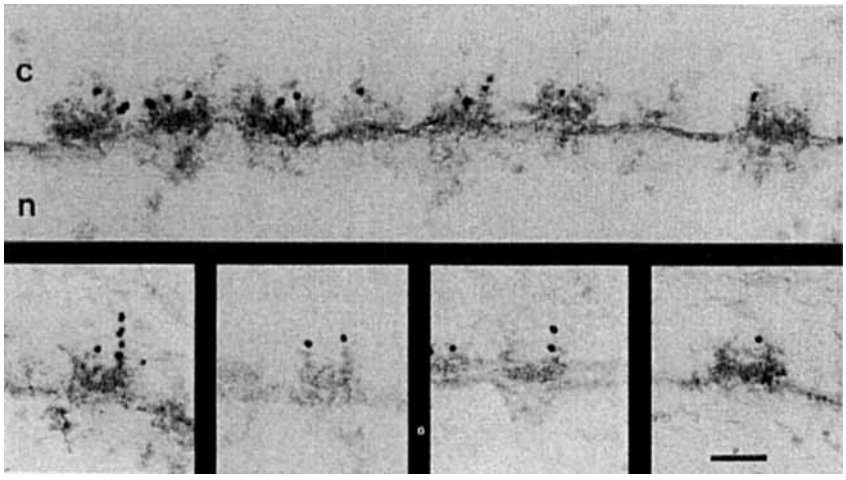

FIGURE 11. CAN/NUP214/p250 is a constituent of the cytoplasmic filaments of the NPC. Triton X-100-treated *Xenopus* oocyte nuclei were labeled with an affinity purified, polyclonal antibody against CAN/NUP214/p250 (Panté et al., 1994) directly conjugated to 8-nm colloidal gold, and prepared for EM by Epon embedding and thin sectioning. Shown is a view along a cross-sectioned NE stretch, together with a gallery of selected examples of gold-labeled NPC cross-sections. These cross-sections show that the anti-CAN/NUP214/p250 antibody exclusively labels the cytoplasmic filaments of the NPC. c, the cytoplasmic side of the NE; n, the nuclear side of the NE. Bar, 100 nm.

Using the monoclonal QE5 antibody, Panté et al. (1994) also identified a distinct NPC subcomplex containing CAN/NUP214/p250 associated with a novel 75-kDa nucleoporin that is not a member of the O-linked NPC glycoprotein family. After detergent/high-salt treatment, these two nucleoporins dissociated from the NPC as a distinct subcomplex, the 'p250-p75 complex', with a molecular mass of at least 1.5 to 2.0 MDa.

iv. RanBP2/NUP358

The small GTPase Ran/TC4 was identified recently as an essential cellular factor required for the import of nuclear proteins (Moore and Blobel, 1993; Melchior et al., 1993) and export of RNA (Moroianu and Blobel, 1995). Therefore, in search of proteins that bind Ran/TC4, a 358-kDa human nucleoporin, termed RanBP2/NUP358, was recently identified and molecularly characterized (Wu et al., 1995; Yokoyama et al., 1995; reviewed by Moore, 1995). Consistent with being a Ran/TC4-binding protein, the amino acid sequence of RanBP2/NUP358 contains four domains homologous to the Ran/TC4-binding protein RanBP1 (Coutavas et al., 1993; Bischoff et al., 1995). These domains, when expressed in *E. coli* and analyzed by gel overlays, bind Ran/TC4·GTP but not Ran/TC4·GDP (Yokoyama et al., 1995). As illustrated in Plate 3, the primary sequence of RanBP2/NUP358 harbors 26 copies of the pentapeptide motif XFXFG diagnostic for the O-linked NPC glycoprotein family (Snow et al., 1987). Based on the reactivity of RanBP2/NUP358 with the monoclonal antibody RL1 whose

epitope includes the O-linked sugar modification common to the members of the O-linked NPC glycoprotein family (Holt et al., 1987), Melchior et al. (1995) concluded that RanBP2/NUP358 is a member of this family. However, in contrast to the other members of this family of NPC glycoproteins, RanBP2/NUP358 does not bind WGA, indicating that the content of O-linked sugar moieties in this protein might be rather low.

In addition to the XFXFG pentapeptide repeat motif, the primary structure of RanBP2/NUP358 also harbors eight zinc finger motifs similar to those of NUP153 (see above and Plate 3), an NH_2-terminal leucine-rich domain containing a leucine zipper motif, and a COOH-terminal domain homologous to cyclophilin (Galat, 1993). The functional significance of these distinct domains remains to be elucidated.

Using three different antibodies, we have immunolocalized RanBP2/NUP358 *in situ* (Yokoyama et al., 1995). As illustrated in Figure 15a, an antibody raised against a fusion protein expressed from the G5 cDNA clone obtained by a yeast two-hybrid screen specifically labeled the cytoplasmic filaments of the NPC. However, few gold labels were also found associated with the nuclear face of the NPC. This nuclear labeling is probably due to the fact that the G5 product contains a region (including the zinc finger motifs) significantly homologous to NUP153, a constituent of the nuclear basket of the NPC (see above and Figure 10). To further establish whether RanBP2/NUP358 is indeed also located at the nuclear face of the NPC, we performed immuno-gold EM with antibodies raised against two different peptides within the G5 sequence: (1) a peptide, termed 551, repeated three times within the RanBP2/NUP358 sequence in the region homologous to NUP153 and (2) the unique 552 peptide located far away from the region homologous to NUP153. As expected, the anti-551 antibody labeled the cytoplasmic filaments of the NPC as well as the nuclear baskets (Figure 12b). In contrast, the anti-552 antibody exclusively labeled the cytoplasmic filaments of the NPC (Figure 12c). Taken together, these immunolabeling data strongly suggest that RanBP2/NUP358 is a constituent of the cytoplasmic filaments of the NPC. This localization has now been confirmed by Wu et al. (1995). The location of RanBP2/NUP358 to the cytoplasmic filaments together with its ability to bind Ran/TC4 points to a functional role of this nucleoporin in the import of nuclear proteins. Most likely, RanBP2/NUP358, possibly in complex with CAN/NUP214/p250 and Tpr/p265 (see below and Figures 11, 13, and Plate 4), represents a constituent of the initial cytoplasmic docking site (i.e., the cytoplasmic filaments) for nuclear proteins during their translocation through the NPC. Consistent with this hypothesis, the anti-G5 antibody inhibits the import of nuclear proteins in a dose-dependent manner (Yokoyama et al., 1995). Moreover, Radu et al. (1995a) reported that RanBP2/NUP358 from rat liver NEs was one of several nucleoporins that binds an NLS-bearing protein in the presence of fractionated cytosol in an overlay assay. More recently, Melchior et al. (1995) demonstrated that Ran/TC4·GTP, but not Ran/TC4·GDP, specifically binds to the cytoplasmic filaments of NPCs in isolated rat liver NEs. Thus, RanBP2/NUP358 might provide the binding sites for both import ligands and Ran/TC4.

In an attempt to molecularly identify NUP180, a 180-kDa nucleoporin previously identified using an autoimmune serum and localized to the cytoplasmic ring and/or filaments of the NPC by immuno-EM (Wilken et al., 1993), Wilken and co-workers cloned a partial cDNA that based on its sequence represents the rat homolog of human

* Plate 4 appears following page 184.

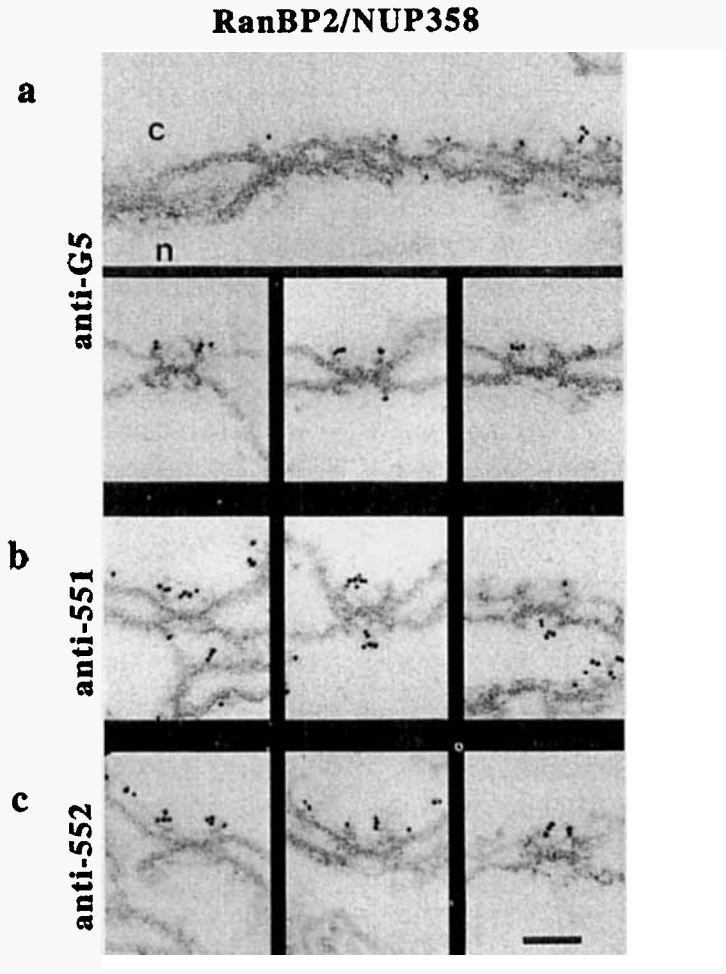

FIGURE 12. RanBP2/NUP358 is a constituent of the cytoplasmic filaments of the NPC. Isolated rat liver NEs were incubated with (**a**) a polyclonal anti-RanBP2/NUP358 antibody that was raised against a fusion protein expressed from the G5 cDNA containing a region homologous to NUP153 (Yokoyama et al., 1995), (**b**) the polyclonal anti-551 peptide antibody that was raised against a peptide repeated three times within the G5 sequence in the region homologous to NUP153, and (**c**) the polyclonal anti-552 peptide antibody that was raised against an unique peptide within the G5 sequence located far away from the region homologous to NUP153. In all three cases, the antibodies were directly conjugated to 8-nm colloidal gold, and the NEs were prepared for EM by Epon embedding and thin sectioning. (**a**) The anti-G5 antibody specifically labels the cytoplasmic filaments of the NPC. However, few gold labels are also associated with the nuclear side of the NPC, due probably to a cross-reaction with NUP153. (**b**) As expected, the anti-551 antibody labels both the cytoplasmic filaments and the nuclear basket of the NPCs. In contrast, the anti-552 antibody exclusively labels the cytoplasmic filaments of the NPCs (**c**). c, the cytoplasmic side of the NE; n, the nuclear side of the NE. Bar, 100 nm (**a**–**c**).

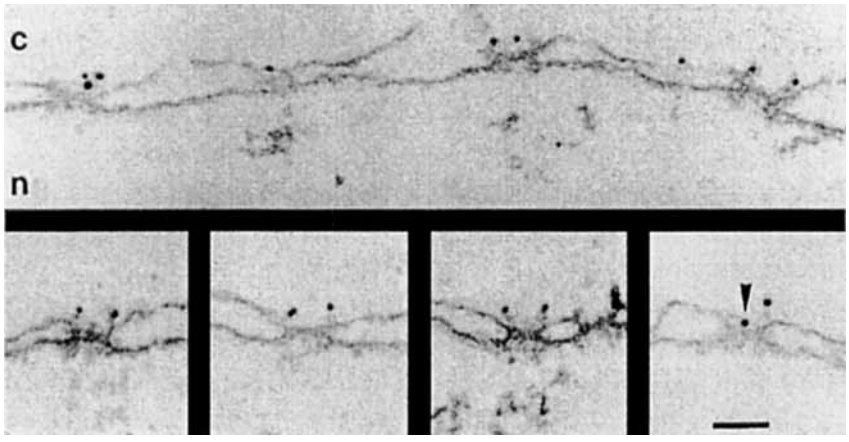

FIGURE 13. Tpr/p265 is a constituent of the cytoplasmic filaments of the NPC. Isolated rat liver NEs were incubated with the monoclonal RL30 antibody, which specifically reacts with rat Tpr/p265 on immunoblots (Byrd et al., 1994), directly conjugated to 8-nm colloidal gold, and prepared for EM by Epon embedding and thin sectioning. Shown is a view along a cross-sectioned NE stretch, together with a gallery of selected examples of gold-labeled NPC cross-sections. These cross-sections reveal that RL30 exclusively labels the cytoplasmic side of the NE. Accordingly, gold particles are associated predominantly with the cytoplasmic filaments of the NPC, which in some cases have bent so as to reach down into the central pore (see arrowhead). c, the cytoplasmic side of the NE; n, the nuclear side of the NE. Bar, 100 nm.

RanBP2/NUP358 (Wilken et al., 1995). Thus, NUP180 as characterized by Wilken et al. (1993) probably represented a proteolytic product of RanBP2/NUP358. Supporting this notion, anti-NUP180 antibodies also reacted with a polypeptide of ~260 kDa (Wilken et al., 1993), which is the apparent molecular weight of RanBP2/NUP358 by SDS-PAGE.

v. NUP98/p97

An O-linked glycoprotein of *Xenopus* egg extracts with an apparent molecular weigh of 97 kDa (estimated from its mobility on SDS-PAGE) has been identified recently and characterized (Powers et al., 1995). A partial amino acid sequence of this protein revealed that p97 is the first vertebrate nucleoporin containing the repetitive GLFG tetrapeptide motif characteristic of a group of yeast nucleoporins (see below and Table 3). The rat homolog of this nucleoporin was cloned and sequenced independently by Radu et al. (1995b) and termed NUP98. As illustrated in Plate 3, the complete amino acid sequence of NUP98/p97 contains 16 GLFG repeats distributed along the NH_2-terminal half of the molecule. In addition to the repetitive GLFG motifs, the amino acid sequence of vertebrate NUP98/p97 is very similar to that of the members of the yeast GLFG nucleoporin family, including an RNA-binding motif at the COOH-terminal end (see below and Plate 5).

The localization of NUP98/p97 has remained elusive. While Radu et al. (1995b) localized this nucleoporin to the nucleo-

plasmic face of the NPC from BRL cells, Powers et al. (1995) have detected NUP98/p97 both at the NE and within the nucleus by immunofluorescence microscopy. Similarly, the possible functional role of NUP98/p97 remains ambiguous. Based on ligand blot assays, Radu et al. (1995b) suggested that this nucleoporin functions as a docking site for NLS-bearing proteins, and they have localized the 'docking function' of the molecule to its NH_2-terminal half that contains the GLFG repetitive motifs. On the other hand, Powers et al. (1995) have shown that *in vitro* reconstituted nuclei lacking NUP98/p97 are competent for the import of nuclear proteins. However, these NUP98/p97-depleted nuclei did not grow and failed to replicate their chromosomal DNA. Thus, NUP98/p97 probably is not essential for the import of nuclear proteins, but instead is somehow involved in DNA replication. Because the localization of this nucleoporin remains ambiguous, it is difficult to explain the phenotype of NUP98/p97–depleted nuclei. Nevertheless, Powers et al. (1995) speculated that if NUP98/p97 were indeed a constituent of the nuclear baskets, DNA replication would take place near or at these distinct NPC structures.

b. Non-O-Linked Peripheral Membrane Proteins

i. NUP107 and NUP155

In rat liver NEs, an additional group of ~30 proteins that do not contain GlcNac, and hence do not bind WGA by WGA-Sepharose affinity chromatography, can be separated from the O-linked NPC glycoproteins and further purified on an SDS-hydroxylapatite column (Radu et al., 1993). Only two of these proteins, NUP107 and NUP155, have been identified as nucleoporins. The genes encoding these two nucleoporins have been cloned and their cDNAs sequenced (Radu et al., 1993, 1994). As illustrated schematically in Plate 3, the deduced amino acid sequences of NUP107 and NUP155 do not reveal any of the repetitive sequence motifs that are a diagnostic feature of the O-linked NPC glycoproteins (see Plate 3 and Table 2). Similar to CAN/NUP214/p250 and RanBP2/NUP358, NUP107 contains a leucine zipper motif at its COOH-terminal end that has been suggested to induce dimerization with a second leucine-zipper-containing polypeptide (Radu et al., 1994). By immuno-EM, anti-peptide antibodies against both NUP107 and NUP155 labeled the NPCs of cultured cells (Radu et al., 1993, 1994). Unfortunately, the preservation of the ultrastructure of the NPCs in the corresponding thin sections is rather poor so that localization of these two proteins to distinct NPC components has not been possible.

ii. Tpr/p265

Tpr (translocated promoter region) was first identified as the product of a human gene encoding a 265-kDa protein whose NH_2-terminal domain appears in oncogenic fusions with the *met*, *trk*, and *raf* proto-oncogenes (Mitchell and Cooper, 1992). Using the monoclonal antibody, RL30, Byrd et al. (1994) identified a 265-kDa nucleoporin in rat liver NEs that, by partial amino acid sequence analysis has been shown to represent the rat homolog of human Tpr. Consistent with being a non-O-linked NPC protein, the amino acid sequence of Tpr lacks the repetitive XFXFG pentapeptide motif diagnostic for the O-linked NPC glycoproteins (Table 2). As illustrated schematically in Plate 3, based on its deduced amino acid sequence, Tpr contains an over-1600-residue-long segment predicted to form

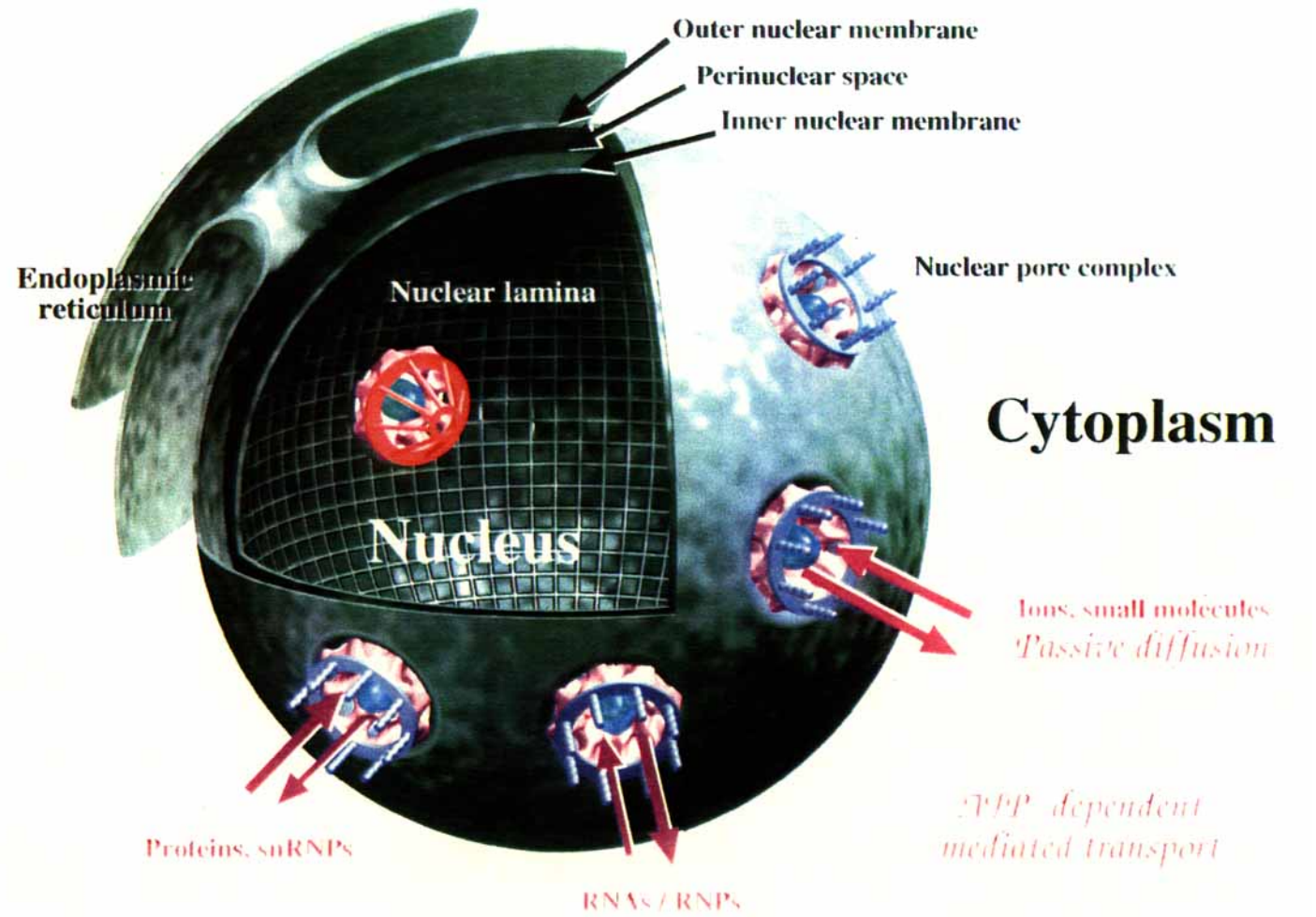

PLATE 1. Schematic representation of the components of the nuclear envelope (NE) and of the molecular trafficking occurring through the nuclear pore complexes (NPCs). The NE consists of an inner and an outer nuclear membrane enclosing the perinuclear space. The outer nuclear membrane faces the cytoplasm and is continuous with the endoplasmic reticulum (ER), thus the perinuclear space of the NE is contiguous with the lumen of the ER. The inner nuclear membrane faces the nucleoplasm, and at its nucleoplasmic surface it is lined by the nuclear lamina (NL), a near-tetragonal meshwork made of intermediate filament-like proteins called nuclear lamins (Aebi et al., 1986; Gerace and Burke, 1988). The NPCs are interposed at irregular intervals between the inner and outer nuclear membrane. Bi-directional molecular trafficking between the nucleus and the cytoplasm occurs through the NPCs, which allows passive diffusion of ions and small molecules and ATP-mediated active transport of proteins, RNAs, and RNPs.

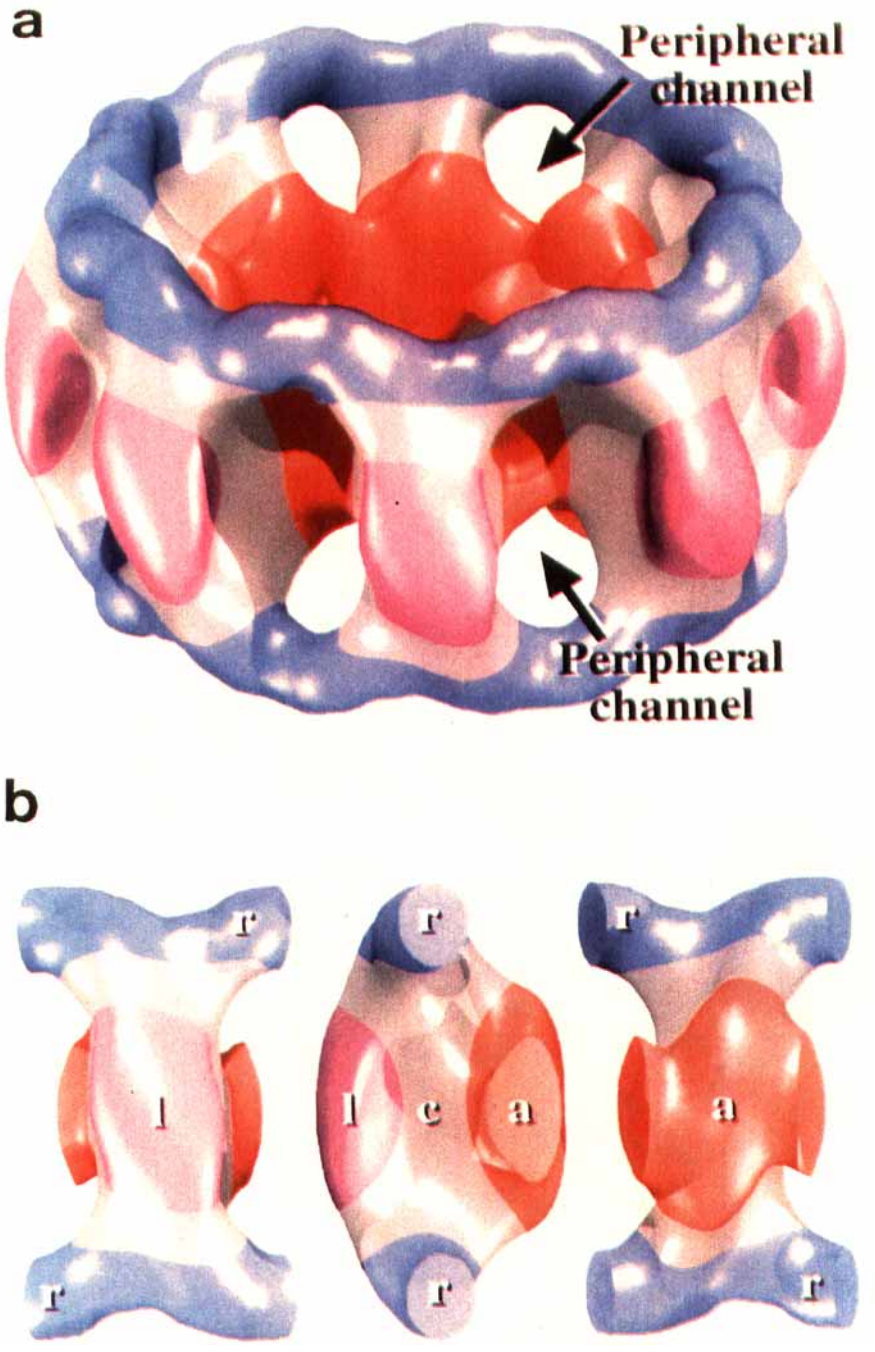

PLATE 2. Surface renderings of the 52-MDa basic framework of the NPC 3-D reconstructed from negatively stained spoke complexes released from the NE after treatment with Triton X-100 (see Figure 2b). (**a**) Slightly forward tilted view of the basic framework that consists of eight multidomain spokes and exhibits strong 822 symmetry, thus indicating that it is built from two identical halves relative to the central plane of the NE. Note that due to its irreproducible appearance from one spoke complex to the next (see Figure 2b), the central channel complex has been omitted in this reconstruction of the basic framework of the NPC. When the pore membrane is positioned in this map, eight ~10-nm-diameter peripheral channels are created between two adjacent spokes and the pore membrane border at a radius of ~40 nm. (**b**) Three different views of one multidomain spoke cut out from the basic framework as shown in (**a**). Accordingly, each multidomain spoke is made of two identical ~3.3-MDa half-spokes that, in turn, are built from four distinct morphological domains, termed annular (a), column (c), lumenal (l), and ring (r) domain. (This figure has been adapted from Hinshaw et al. (1992).)

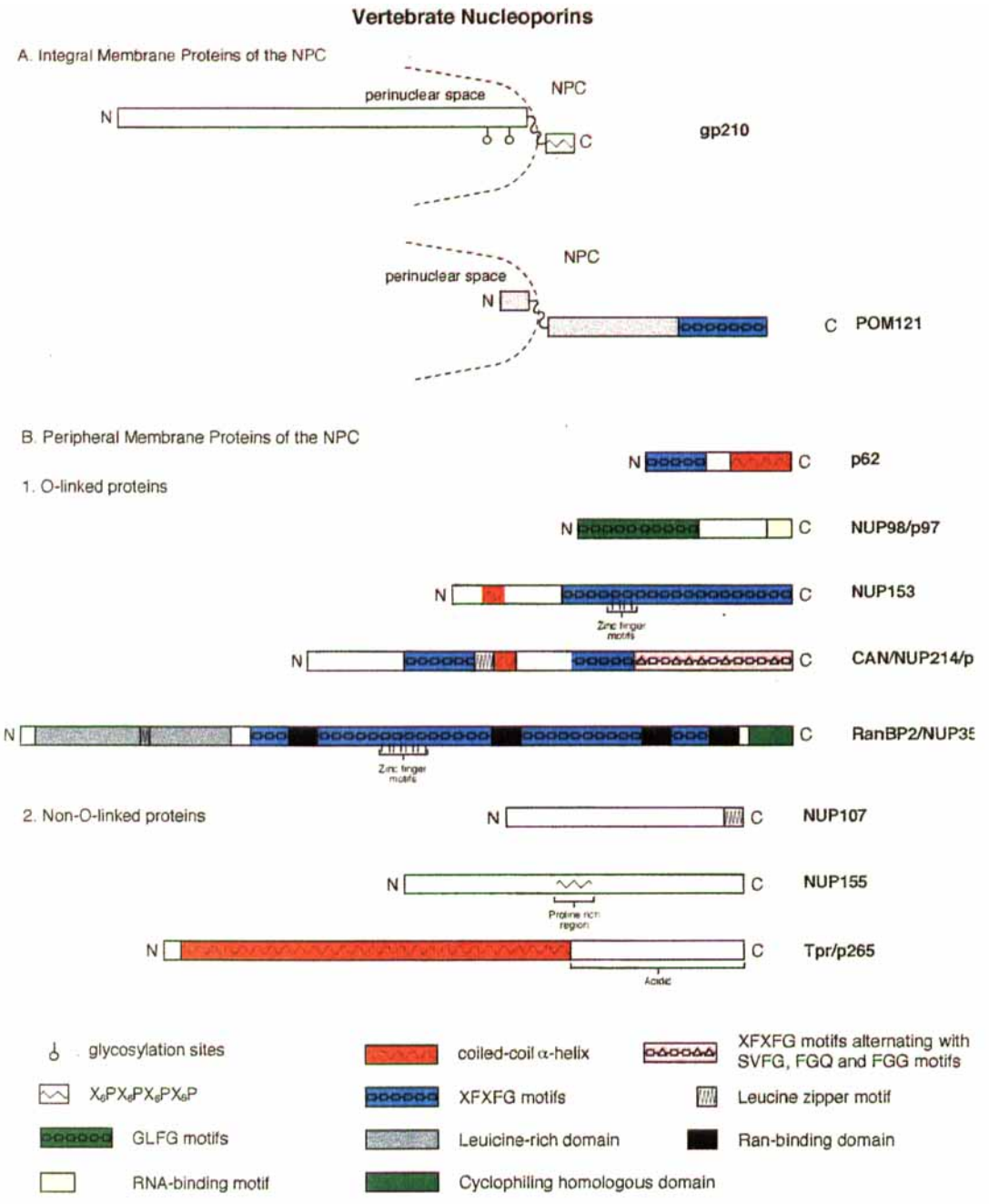

PLATE 3. Schematic diagram of the cloned and sequenced vertebrate nucleoporins according to their amino acid sequences. (**A**) Two integral membrane proteins of the NPC, gp210 and POM121, have thus far been cloned and sequenced. They both reveal a distinct stretch of hydrophobic amino acids that is predicted to represent a transmembrane segment traversing the pore membrane. gp210 contains a large NH_2-terminal domain residing in the lumen of the NE, and a small COOH-terminal tail associated with the basic framework of the NPC. In contrast, POM121 consists of a short NH_2-terminal domain residing in the lumen of the NE, and a long NPC associated COOH-terminal domain that contains the repetitive XFXFG pentapeptide motifs. The N-linked glycosylated residues of gp210 occur in the lumenal domain close to the nuclear membrane (Greber et al., 1990). As documented in **B**, depending on their content of O-linked *N*-acetylglucosamine (GlcNac) residues, two families of peripheral membrane proteins of the NPC have been distinguished: (1) O-linked NPC glycoproteins that contain several copies of the pentapeptide motif XFXFG or the tetrapeptide motif GLFG, and (2) non-O-linked NPC proteins that do not contain any repetitive sequence motifs. In addition, p62 contains a COOH-terminal α-helical coiled-coil domain, and NUP153 and RanBP2/NUP358 harbor zinc finger motifs. In the case of CAN/NUP214/p250, the repetitive XFXFG motif alternates with repetitive SVFG, FGQ, and FGG motifs. The amino acid sequences of the three members of the non-O-linked NPC protein family are unique. In the case of Tpr/p265, it contains a ~1600-residues-long α-helical coiled-coil domain near its NH_2-terminal end. For more information about these proteins, see Table 2 and references therein.

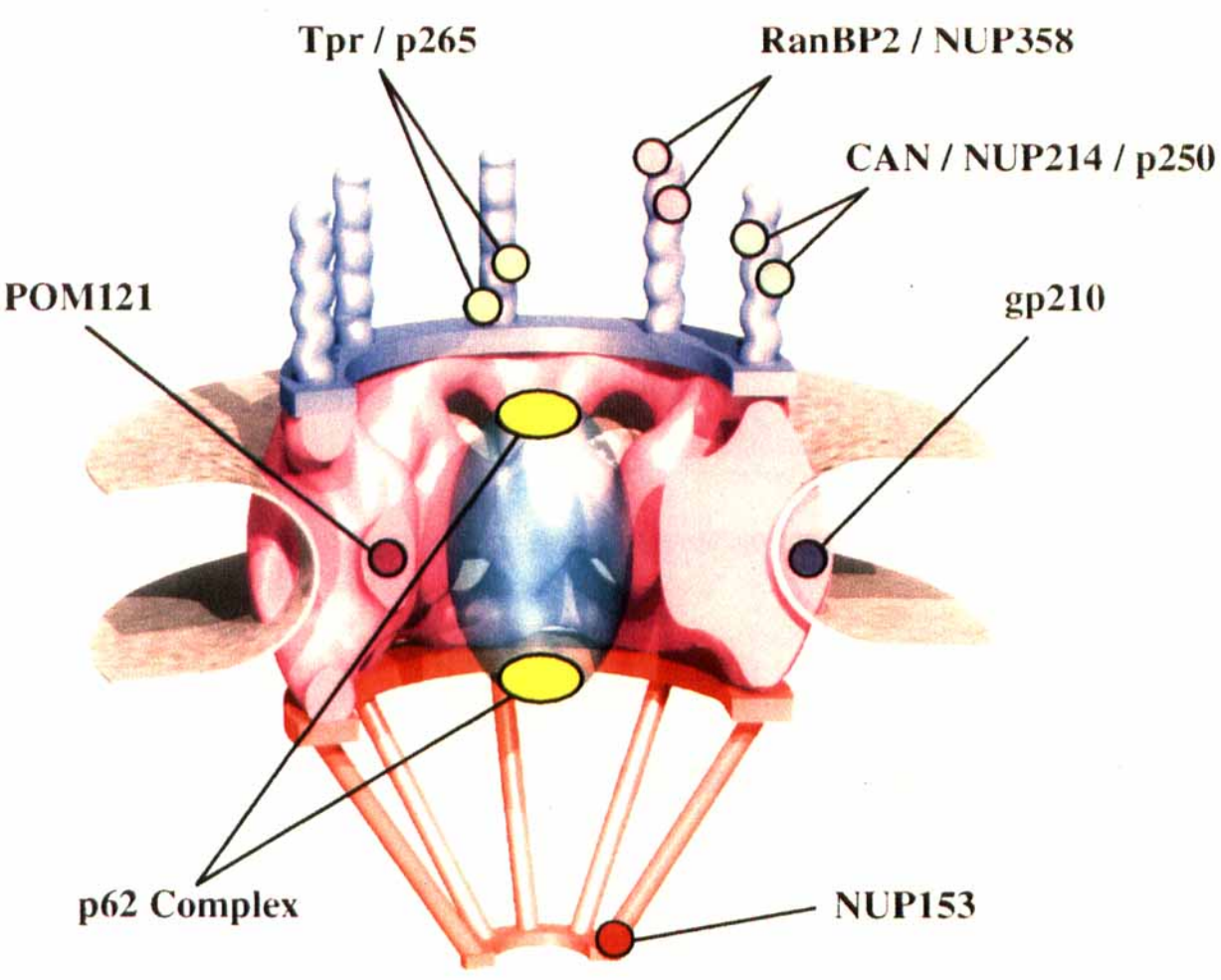

PLATE 4. Schematic diagram summarizing the major structural components of the NPC together with the immuno-localization of five nucleoporin epitopes within the 3-D architecture of the NPC. The major structural components of the NPC include the basic framework (i.e., the spoke complex) in pink, the central plug or channel complex in translucent light blue, the cytoplasmic ring and the cytoplasmic filaments in blue, and the nuclear ring and nuclear basket in orange. The 52-MDa basic framework of the NPC has been adapted from a random conical tilt reconstruction of negatively stained detergent-released NPCs (Hinshaw et al., 1992). In this consensus model of the NPC, we have also pictured a cytoplasmic and a nuclear ring in addition to the two tenuous rings defined by the ring domain of the multidomain spoke complex (see Plate 2a; Hinshaw et al., 1992). The cytoplasmic filaments and nuclear basket have been modeled based on EM data obtained by Ris (1991), Jarnik and Aebi (1991), and Goldberg and Allen (1992). The central plug or channel complex has been modeled as a translucent ellipsoidal particle to indicate the fact that its definite structure remains elusive. Epitopes of five different nucleoporins are marked in this model: whereas CAN/NUP214/p250, Tpr/p265, and RanBP2/NUP358 exhibit epitopes residing in the cytoplasmic filaments (see also Figures 11, 12, and 13; Byrd et al., 1994; Panté et al., 1994; Yokoyama et al., 1995), NUP153 exhibits an epitope near or at the terminal ring of the nuclear basket (see Figure 10; Panté et al., 1994), and p62 epitopes are residing near or at both the cytoplasmic and nuclear face of the central plug or channel complex (see Figure 9; Guan et al., 1995). Epitopes for the transmembrane glycoproteins gp210 and POM121 are also indicated. Based on topological studies, most of the gp210 mass is predicted to reside in the lumen of the double membrane of the NE (Greber et al., 1990). In contrast, most of the mass of POM121 has been localized by immuno-EM within the NPC proper (Söderqvist and Hallberg, 1994).

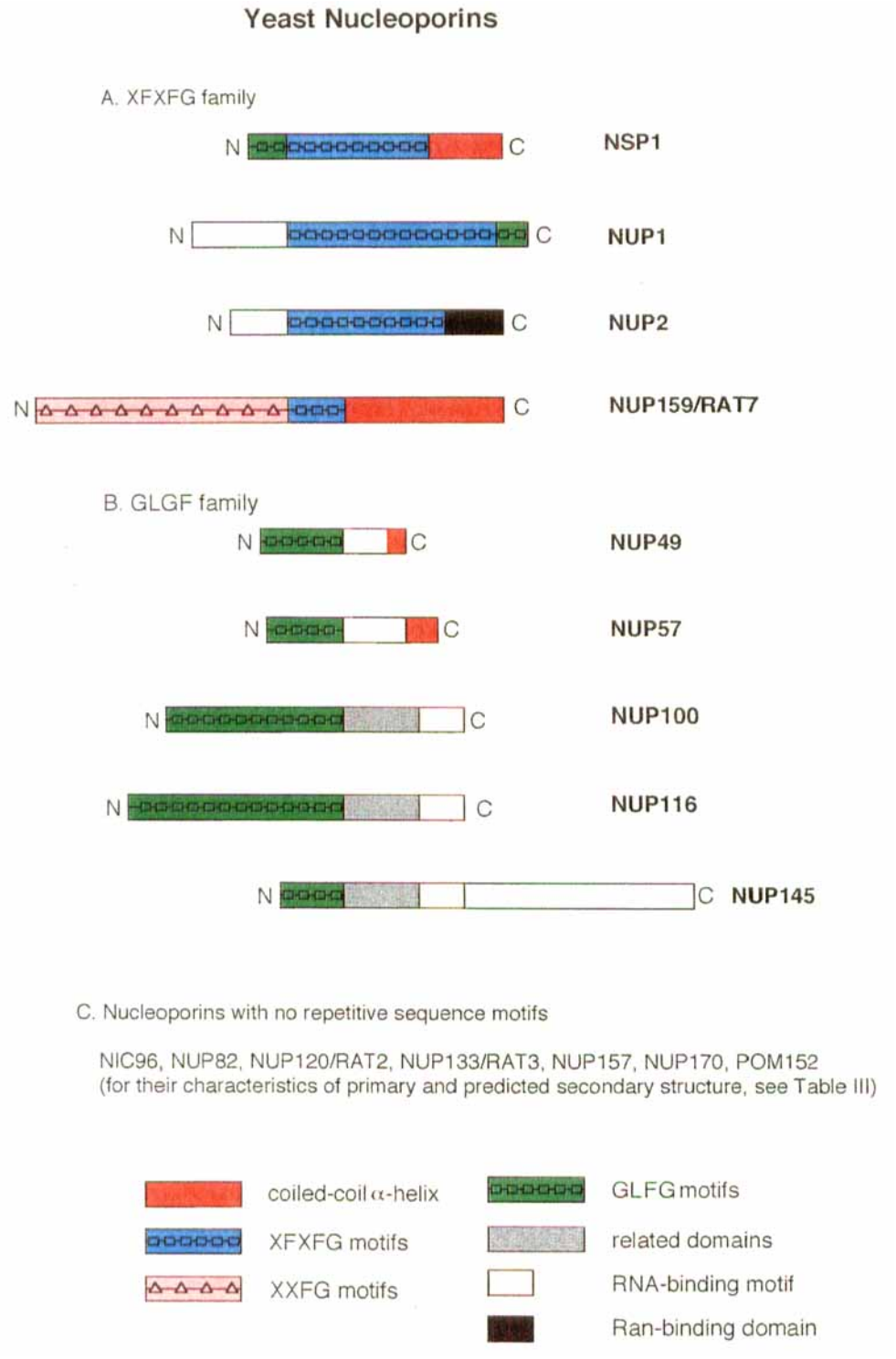

PLATE 5. Schematic diagram of the cloned and sequenced yeast nucleoporins according to their amino acid sequences. The known yeast nucleoporins are grouped into two families depending on the occurrence of highly repetitive sequence motifs: (**A**) the XFXFG family, which contains several copies of a more or less degenerate XFXFG pentapeptide motif clustered in the central part of each protein; (**B**) the GLFG family, whose members contain several copies of a more or less degenerate GLFG tetrapeptide motif within their NH_2-terminal domain. (**C**) In addition, seven yeast nucleoporins that do not reveal any repetitive sequence motifs have been identified recently and molecularly characterized. For more information about these proteins, see Table 3 and References therein.

an α-helical coiled-coil via its heptad repeats (Mitchell and Cooper, 1992).

As illustrated in Figure 13, immuno-EM with the RL30 antibody indicates that Tpr/p265 is a constituent of the cytoplasmic filaments. Thus, at least two nucleoporins, CAN/NUP214/p250 and Tpr/p265, both having epitopes mapping to the cytoplasmic filaments (Figures 11 and 13), are involved in oncogenesis. The functional significance of this apparent coincidence, however, remains elusive. The localization of Tpr to the cytoplasmic filaments together with its long-predicted α-helical coiled-coil region suggests that this protein might form the backbone of the cytoplasmic filaments (Byrd et al., 1994).

B. Toward the Molecular Architecture of the NPC

As displayed schematically in Plate 4, we now have immunolocalized epitopes of five different nucleoporins (i.e., p62, NUP153, CAN/NUP214/p250, RanBP2/NUP358, and Tpr/p265) within the 3-D architecture of the NPC. With the exception of p62, which is associated with both the cytoplasmic and nuclear face of the central channel complex, these nucleoporins are constituents of the peripheral components of the NPC: NUP153 is a constituent of the nuclear baskets displaying epitopes near or at its terminal ring, whereas CAN/NUP214/p250, RanBP2/NUP358 and Tpr/p265 are constituents of the cytoplasmic filaments of the NPC.

As can be appreciated in Plate 4, with the exception of the transmembrane glycoproteins gp210 and POM121, the nucleoporin epitopes that have thus far been identified with distinct structural components of the NPC are localized at either the cytoplasmic or nuclear periphery of the NPC. Therefore, the molecular architecture of the 52-MDa basic framework of the NPC (i.e., the spoke complex; see Plate 2a) remains elusive. Thus far, gp210 and POM121 are the only two nucleoporins which have been identified as constituents of the basic framework of the NPC (Greber et al., 1990; Söderqvist and Hallberg, 1994).

C. Yeast Nucleoporins

As with vertebrate nucleoporins, immunological procedures were initially employed to identify yeast nucleoporins. NSP1 was the first yeast nucleoporin to be molecularly characterized using antibodies against a yeast nucleoskeletal preparation (Hurt, 1988). Subsequently, several yeast nucleoporins were identified and molecularly characterized by screening yeast expression libraries with antibodies raised against rat liver NEs (i.e., NUP1, NUP2, NUP49, NUP100, NUP116, and NUP145) (Davis and Fink, 1990; Wente et al., 1992; Loeb et al., 1993; Wente and Blobel, 1994).

Recently, the use of three distinct experimental approaches has led to the identification and molecular characterization of an ever increasing number of yeast nucleoporins (Table 3). The first approach was pioneered by Hurt's group and consists of using a synthetic lethal screen to select genes whose products interact with a known nucleoporin (reviewed by Doye and Hurt, 1995). These synthetic lethal screens are generated by combining a mutant form of a gene encoding a known nucleoporin, such as NSP1, that yields viable cells with another mutated gene that functionally overlaps with the first gene, thereby causing cell death or synthetic lethality. The newly isolated gene may then be used to identify

other genes encoding new proteins, and so on. Thus, in principle, this genetic approach should allow identification of most of the yeast nucleoporins. In fact, using this approach the genes encoding eight yeast nucleoporins have thus far been isolated (i.e., NIC96, NUP49, NUP57, NUP82, NUP116, NUP133, NUP145, and NUP170) (Wimmer et al., 1992; Grandi et al., 1993; Doye et al., 1994; Fabre et al., 1994; Aitchison et al., 1995a; Grandi et al., 1995a,b). Obviously, because any protein interacting with a nucleoporin (e.g., cellular factors mediating nucleocytoplasmic transport) might be isolated using such a synthetic lethal screen, a thus identified protein might not necessarily represent a bone fide (i.e., integral) constituent of the NPC. This was the case with SRP1, the yeast homologue of importin α (Görlich et al., 1994), which was identified in a synthetic lethal screen based on a NUP1–disrupted strain (Belanger et al., 1994). Therefore, to conclude that the product of a gene isolated by a synthetic lethal screen using a nucleoporin as the 'bait' is indeed a bone fide nucleoporin, additional data is required such as immuno-EM. Nevertheless, this genetic approach has allowed not only the identification of new yeast nucleoporins, but has also identified subcomplexes of nucleoporins (Grandi et al., 1993; 1995a,b).

The second approach that has been employed to identify yeast nucleoporins takes advantage of the procedure recently developed by Rout and Blobel (1993) to bulk isolate NPCs from yeast. By this isolation procedure, a preparation containing over 80 uncharacterized proteins that are candidate nucleoporins has been obtained. By peptide sequencing of the polypeptides separated by SDS-PAGE, six of these proteins were recently identified as nucleoporins: NUP82, NUP120/RAT2, NUP133/RAT3, NUP157, NUP159/RAT7, and POM152 (Wozniak et al., 1994; Aitchison et al., 1995a;b; Hurwitz and Blobel, 1995; Kraemer et al., 1995; Pemberton et al., 1995). Thus, this isolation procedure has opened the possibility to more systematically identify the protein constituents of yeast NPCs, so it is expected that many more yeast nucleoporins will be identified in the near future by this approach. Most importantly, the yeast system offers the possibility to combine molecular genetics approaches with biochemical, structural, and functional analyses of the NPC.

Finally, the third approach used to identify yeast nucleoporins is based on the identification of genes whose products are required for the nuclear export of mRNA in yeast. Accordingly, screens of yeast temperature-sensitive mutants that accumulate poly(A)$^+$RNA in the nucleus at the restrictive temperature have been developed (Amberg et al., 1992; Kadowaki et al., 1992, 1994). Several genes, termed ribonucleic acid trafficking or *rat* (Amberg et al., 1992) and mRNA transport or *mtr* (Kadowaki et al., 1992, 1994), have been obtained by the use of these screens. As yet, however, most of the products of these genes have remained uncharacterized. Obviously, the proteins encoded by these genes should somehow be involved in different steps along the mRNA export pathway, but without *a priori* being bona fide nucleoporins. For example, RAT1, the product of the first characterized gene identified by this approach, has been a 116-kDa nuclear protein (Amberg et al., 1992). Nevertheless, because the NPC does play a central role in mRNA export, it is expected that at least some of the genes identified by these screens will indeed encode bone fide yeast nucleoporins. In fact, three new nucleoporins, NUP120/RAT2, NUP133/RAT3, and NUP159/RAT7, have been identified recently by this approach (Gorsch et al., 1995; Heath et al., 1995; Li et al., 1995). Moreover, this approach has the attractive

property that the thus identified nucleoporins will exhibit specific transport functions.

The yeast nucleoporins identified by the three different experimental approaches discussed above can be classified into three groups based on the occurrence of highly repetitive sequence motifs (Table 3 and Plate 5). Accordingly, the first group represents the XFXFG family which contains several copies of a more or less degenerate XFXFG pentapeptide motif clustered in the central part of each protein (Plate 5). Whereas this XFXFG motif is a diagnostic feature for the vertebrate O-linked NPC glycoproteins (see above and Plate 3), the corresponding yeast nucleoporins appear not to be glycosylated (Kalinich and Douglas, 1989). Moreover, no obvious relationship has yet been depicted between these vertebrate and yeast nucleoporins. So far, the only exception is NSP1, which has been considered to be the yeast homolog of vertebrate p62: both proteins have the same domain structure (see Plate 3 and Plate 5) with 50% sequence similarity in their COOH-terminal domains (Carmo-Fonseca et al., 1991; Fabre and Hurt, 1994). Members of this yeast XFXFG family include NSP1, NUP1, NUP2, and NUP159/RAT7 (Hurt, 1988; Davis and Fink, 1990; Loeb et al., 1993; Gorsch et al., 1995).

A second group of yeast nucleoporins represents the GLFG family whose members contain multiple copies of a degenerate GLFG tetrapeptide motif within their NH_2-terminal domain (Plate 5). Members of this family include NUP49, NUP57, NUP100, NUP116 and NUP145 (Wente et al., 1992; Wimmer et al., 1992; Fabre et al., 1994; Wente and Blobel, 1994; Grandi et al., 1995b).

Finally, the third group includes yeast nucleoporins that do not contain any repetitive sequence motifs. Members of this group are NIC96, NUP82, NUP120/RAT2, NUP133/RAT3, NUP157, NUP170, and POM152 (Grandi et al., 1993, 1995a; Doye et al., 1994; Wozniak et al., 1994; Aitchison et al., 1995a,b; Heath et al., 1995; Hurwitz and Blobel, 1995; Li et al., 1995; Pemberton et al., 1995).

Although all these yeast nucleoporins have been localized to the nuclear periphery by indirect immunofluorescence, as yet the precise localization within the 3-D NPC architecture has remained elusive. In the following three sections, we summarize the structural and functional properties of these three groups of yeast nucleoporins (see also reviews by Fabre and Hurt, 1994; Panté and Aebi, 1994b; Bastos et al., 1995; Davis, 1995).

a.The XFXFG Family

Thus far, four members of the XFXFG family of yeast nucleoporins have been cloned and sequenced (Table 3). As illustrated in Plate 5, the most striking feature of the predicted amino acid sequence of these four nucleoporins is a central region containing multiple copies of a more or less degenerate XFXFG pentapeptide motif that is separated by highly charged spacer sequences. This motif is less abundant in NUP159/RAT7, in which it is present only three times, compared with NSP1 and NUP1, in which the XFXFG motif is repeated more than 20 times. Although Radu et al. (1995b) have speculated that the repetitive motif of these nucleoporins might have a functional role, the XFXFG motifs of NSP1 appear not to be essential for function (Nehrbass et al., 1990). Accordingly, when expressed at authentic expression levels, the COOH-terminal domain of recombinant NSP1, which does not contain any repetitive XFXFG motifs, is sufficient to complement a mutant lacking NSP1. Furthermore, a temperature-sensitive mutation in this domain arrested cell growth and prevented accumulation of

a nucleolar protein (Nehrbass et al., 1990; Hurt, 1990).

With the exception of the COOH-terminal domains of NSP1 and NUP159/RAT7 both of which contain heptad repeats and hence are predicted to form α-helical coil-coils (Plate 5), neither the NH_2- nor the COOH-terminal domains of these nucleoporins share any common features. Interestingly, the COOH-terminal domain of NUP2 exhibits a sequence similarity to the Ran/TC4-binding protein RanBP1 (Coutavas et al., 1993; Bischoff et al., 1995). Accordingly, this domain interacts with Ran/TC4 in both a blot assay and the two-hybrid system (Dingwall et al., 1995). Because Ran/TC4 is required for the import of nuclear proteins (Moore and Blobel, 1993; Melchior et al., 1993), it has been suggested that NUP2 might have a direct role in nucleocytoplasmic transport (Dingwall et al., 1995). Moreover, both NUP1 and NUP2 bind SRP1 (Belanger et al., 1994), the yeast homolog of importin α (Görlich et al., 1994). Thus, these nucleoporins might be components of the docking sites for import ligands in yeast NPCs. As yet, however, neither NUP1 nor NUP2 have been localized within the 3-D structure of the NPC; hence, the proposed functional role for NUP1 and NUP2 is still speculative.

The functional roles of the other members of the XFXFG family of yeast nucleoporins have been studied by phenotypic analysis of yeast strains containing mutations in each of the genes encoding these nucleoporins (Mutvei et al., 1992; Bogerd et al., 1994; Gorsch et al., 1995). Accordingly, mutations in three of these nucleoporin genes cause defects in NPC function and alterations of the NE morphology and NPC distribution in different ways. (1) Depletion of NSP1 inhibits the import of nuclear proteins and decreases the density of NPCs (Mutvei et al., 1992). (2) NUP1 mutant cells exhibit both import and export defects, and their NEs form long projections extending into the cytoplasm (Bogerd et al., 1994). (3) Mutations in the gene encoding the nucleoporin NUP159/RAT7 cause inhibition of mRNA export and NPC clustering within one region of the NE (Gorsch et al., 1995). Because the exact localization of these nucleoporins within the 3-D NPC architecture remains elusive, it is difficult to explain these phenotypes. Nevertheless, it has been speculated that NPC clustering might be due to altered interactions of the NPCs with cytoskeletal or nuclear structures (Gorsch et al., 1995).

Affinity chromatography using the COOH-terminal domain of NSP1 fused to protein A revealed that this nucleoporin forms an essential NPC subcomplex, 'the NSP1 complex', containing in addition to NSP1 the yeast nucleoporins NUP49, NUP57, and NIC96 (see below; Grandi et al., 1993, 1995b). As the amino acid sequence of NSP1 is somewhat homologous to p62, the NSP1 complex might represent the yeast homolog of the mammalian p62 complex (see above). However, in contrast to the mammalian p62 complex (Finlay et al., 1991; Kita et al., 1993; Buss and Stewart, 1995; Guan et al., 1995), as yet comparably little is known about the molar stoichiometry, molecular interactions, localization within the NPC, and possible function of the NSP1 complex.

2. The GLFG Family

As documented in Table 3 and Plate 5, to date five members of the GLFG family of yeast nucleoporins have been cloned and sequenced. Accordingly, these contain highly homologous sequence segments, including an NH_2-terminal domain with repetitive GLFG tetrapeptide motifs separated by essentially uncharged spacers. In addi-

tion, the COOH-terminal domain of both NUP49 and NUP57 contains heptad repeats giving rise to a α-helical coiled-coil conformation. It has been proposed that these a-helical coiled-coils are involved in the interaction of these nucleoporins to form the NSP1 complex (Grandi et al., 1995b).

An interesting feature of the amino acid sequence of three of these nucleoporins, NUP100, NUP116, and NUP145, is the presence of an octapeptide motif also found in RNA-binding proteins (Plate 5; Fabre et al., 1994). When fragments of NUP116 and NUP145 containing this RNA-binding motif were expressed as fusion proteins in *E. coli*, the fusion proteins were found to bind RNA *in vitro* (Fabre et al., 1994). Based on these results, it has been suggested that NUP116 and NUP145 may be involved in RNA recognition and/or may play a role in RNA translocation through the NPC (Fabre and Hurt, 1994; Fabre et al., 1994).

Using mutant strains, it has been demonstrated that NUP49, NUP57, and NUP145, but not NUP100 and NUP116, are essential for yeast cell growth (Wente et al., 1992; Wimmer et al., 1992; Fabre et al., 1994; Wente and Blobel, 1994; Grandi et al., 1995b). Therefore, some of these mutants have been examined in the EM to determine whether they perturb the structure or distribution of the NPCs and/or the organization or morphology of the NE (Wente and Blobel, 1993, 1994). It was found that a membrane seal was formed over the cytoplasmic face of the NPCs of the NUP116-deficient yeast cells which, while still permitting nuclear export of polyadenylated RNA to occur, caused the export substrate to accumulate within the cytoplasmic membrane herniations covering the NPCs (Wente and Blobel, 1993). Similarly, deletion/disruption of the NH_2-terminal end of NUP145 yielded yeast nuclei with clusters of numerous NPCs interconnected by a network of NE herniations (Wente and Blobel, 1994). Based on these findings, it has been proposed that NUP116 and NUP145 are possibly involved in establishing specific NPC-NE interactions and/or mediating NPC biogenesis (Wente and Blobel, 1993, 1994).

More recently, Iovine et al. (1995) demonstrated that the repetitive GLFG motifs of NUP116 are involved in NPC function. Accordingly, deletion of the NH_2-terminal domain of NUP116 containing the GLFG repetitive tetrapeptide motif inhibits the import of nuclear proteins. Moreover, overexpression of NUP116 blocks RNA export. Also, the GLFG region of NUP116, but not that of the other four members of the GLFG family, is required for yeast cell viability. Therefore, it appears that not all GLFG regions are functionally equivalent. Iovine et al. (1995) also found that the GLFG region of NUP116 interacts with Kap95 (Enenkel et al., 1995), the yeast homolog of importin-β (Adam and Adam, 1994; Görlich et al., 1995), in both an import ligand blot assay and the two-hybrid system. The GLFG regions of NUP100 and NUP145 also bind Kap95 in a ligand blot assay; however, these nucleoporins do not play an essential role in NPC function *in vivo*. Therefore, *in vitro* ligand blot assays are not necessarily sufficient to demonstrate functional roles for nucleoporins.

c. Other Yeast Nucleoporins

Seven additional yeast nucleoporins have been identified recently (Table 3). None of these contain repetitive sequence motifs, nor do they share any sequence similarities with other nucleoporins. One of these, POM152, is an integral membrane protein (Wozniak et al., 1994) that was identified by using the isolation procedure for yeast NPCs (Rout and Blobel, 1993) in combination with the biochemical approach used to

identify gp210 (Gerace et al., 1982). Like gp210, POM152 reacts with ConA. However, the deduced amino acid sequence of POM152 does not share any similarity with either gp210 or POM121, except for a 19-residue-long segment near its NH_2-terminal domain that is similar to a corresponding segment of POM121. Analysis of the amino acid sequence of POM152 indicated that this protein contains a 20-residue-long transmembrane domain between residues 175 and 196. Because the COOH-terminal domain (residues 196 to 1337) of POM152 contains three putative sites for N-linked glycosylation with at least one of them being actually glycosylated, in analogy to gp210 (see above), this domain has been proposed to reside in the lumen of the double membrane of the NE. However, the exact topology of this protein remains to be established.

In an attempt to more clearly characterize the NSP1 complex, two nucleoporins of molecular weight 96 and 82 kDa were identified recently by affinity chromatography purification of yeast proteins interacting with the COOH-terminal domain of NSP1 tagged with protein A (Grandi et al., 1993, 1995a). The genes encoding these two nucleoporins were cloned via short amino acid sequences determined from the purified proteins. Their deduced amino acid sequences revealed that these two proteins do not contain either XFXFG or GLFG repetitive motifs. However, based on indirect immunofluorescence microscopy, these two proteins were classified as nucleoporins (Grandi et al., 1993; 1995a; Hurwitz and Blobel, 1995). The 96-kDa protein, termed NIC96 for nucleoporin interacting component of 96 kDa, was found in a complex with NSP1, NUP57, and NUP49 (the NSP1 complex). The NH_2-terminal domain of NIC96 contains heptad repeats giving rise to a α-helical coiled-coil formation. As similar heptad repeats are also present in the COOH-terminal domains of the other members of the NSP1 complex, it has been speculated that coiled-coil interactions between these nucleoporins represent the structural basis for their mutual interactions within the NSP1 complex (Grandi et al., 1993; 1995b). In support of this hypothesis, deletions in the COOH-terminal domain of NIC96 impair interaction with a core complex consisting of NSP1, NUP57, and NUP49 (Grandi et al., 1995b). Moreover, deletion of NIC96 causes inhibition of nuclear uptake of NLS-containing proteins, but it does not affect the export of mRNA. Thus, similar to the p62 complex (see above; Finlay et al., 1991; Kita et al., 1993; Buss and Stewart, 1995; Guan et al., 1995), based on these findings, it has been proposed that the NSP1 complex is required for the import of nuclear proteins through the NPC.

Using the yeast NPC purification procedure (Rout and Blobel, 1993), the 82-kDa nucleoporin that also interacts with NSP1, termed NUP82, has been molecularly characterized by another group (Hurwitz and Blobel, 1995). Similar to NSP1, NUP49, and NUP57, NUP82 also contains heptad repeats within its COOH-terminal domain that might mediate interaction with NSP1 via α-helical coiled-coil formation. However, NUP82 is not present in the NSP1 complex containing NIC96 (see above); hence, it probably forms a distinct complex with NSP1 (Grandi et al., 1995a). Yeast cells depleted of NUP82 are defective for poly(A)$^+$RNA export (Grandi et al., 1995a; Hurwitz and Blobel, 1995). Taken together, NSP1 seems to be associated with two distinct complexes, one containing NIC96 and the other NUP82, that are involved in different transport pathways through the NPC.

Using three different experimental approaches, several researchers recently identified and molecularly characterized the nucleoporin NUP133/RAT3 (Doye et al., 1994, Li et al., 1995; Pemberton et al., 1995).

Amino acid sequence analysis of NUP133/RAT3 did not reveal any distinct structural features except for two 16– to 18-residue-long hydrophobic stretches (Doye et al., 1994). The gene encoding NUP133/RAT3 is not essential for yeast cell viability. Cells in which this gene has been disrupted accumulate poly(A)$^+$RNA in the nucleus, but without affecting the import of nuclear proteins (Doye et al., 1994, Li et al., 1995; Pemberton et al., 1995). In addition, these mutant yeast cells reveal clustering of NPCs within the NE, indicating that NUP133/RAT3 might be involved in NPC biogenesis (see above; Li et al., 1995).

Using a POM152 synthetic lethal screen, a gene encoding a 170-kDa nucleoporin termed NUP170 was cloned recently and sequenced (Atchison et al., 1995a). This nucleoporin is present in an amount similar to that of POM152 in a yeast NPC preparation that also contains a 160-kDa protein. The latter protein was molecularly characterized by direct microsequencing (Atchison et al., 1995a). Because its amino acid sequence revealed a molecular mass of 157 kDa, this nucleoporin was termed NUP157. Interestingly, the primary structures of NUP157 and NUP170 are similar to each other and also to the vertebrate nucleoporin NUP155 (see above; Radu et al., 1993). However, NUP157 and NUP170 appear to be functionally distinct: while NUP170 mutants yield synthetic lethality with POM152 mutants, NUP157 mutants do not. Depletion or overexpression of NUP170 causes abnormalities in the morphology of the NE. Thus, similar to NUP133/RAT3 (see above; Li et al., 1995), NUP170 might play some role in NPC biogenesis.

Finally, the gene encoding the nucleoporin NUP120/RAT2 was been identified independently recently by two groups (Aitchison et al., 1995b; Heath et al., 1995). The amino acid sequence of this nucleoporin exhibits similarity to NUP133/RAT3. Sequence analysis revealed the presence of two leucine zipper motifs that might be involved in protein-protein interactions within the NPC. Deletion of the *nup120/rat2* gene causes poly(A)$^+$RNA nuclear accumulation, clustering of NPCs within one region of the NE, and nucleolar fragmentation (Aitchison et al., 1995b; Heath et al., 1995).

V. CONCLUDING REMARKS

In the last few years significant advances have been made in elucidating the 3-D architecture of the NPC. As documented in Plate 2 and Plate 4, a series of electron microscopy studies involving a variety of specimen preparation methods have yielded a consensus model of the NPC and its major components. However, as most of this structural information stems from studies where the nuclear membranes have been solubilized, a step most likely causing more or less serious structural alterations, the 3-D architecture of the native NPC remains to be determined. Nevertheless, structural studies of the NPC are now entering a phase in which it should be possible to monitor structural changes associated with nucleocytoplasmic transport.

Toward deciphering the molecular composition of the 120-MDa NPC, to date over 20 nucleoporins have been identified, characterized, and cloned and sequenced. In particular, the number of yeast nucleoporins is increasing almost weekly. In addition, several nucleoporins have now been located to distinct structural components of the NPC by immuno-EM (see Figures 9 to 13 and Plate 4). Hence, the dissection of the *molecular* architecture of the NPC is well under way. However, as evident from Plate 4, the nucleoporins thus far identified, which represent about 15% of the entire NPC mass, are almost exclusively constituents of the

cytoplasmic and nuclear periphery (i.e., the cytoplasmic filaments and the nuclear basket) of the NPC (see Plate 4). Therefore, we have to go a long way to decipher the *molecular* architecture of the basic framework of the NPC. Furthermore, relatively little is known about the 3-D structure and native conformation of these nucleoporins, their mutual interactions, and their interactions with transport ligands and cellular factors involved in nucleocytoplasmic transport, and hence their functional roles in terms of the distinct steps implicated in protein and RNA import/export. Nevertheless, genetic and biochemical investigations are beginning to identify and characterize distinct NPC subcomplexes, and to define the role(s) these may play in mediated transport. With this information in hand, it is becoming feasible to reconstitute distinct NPC components or subcomplexes, and eventually the entire NPC, *in vitro*. Last but not least, major advances are being made in identifying, characterizing, and cloning and sequencing the cytosolic and nuclear factors that via their coordinated spatial and temporal interactions with transport ligands and specific nucleoporins, are defining the signal pathways for nuclear import and export through the NPC.

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