Mini-Review

The Nuclear Pore Complex

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MOLECULAR trafficking between the nucleus and the cytoplasm of interphase eukaryotic cells occurs via the nuclear pore complexes (NPCs),[1] organelles with a roughly cylindrical shape (~120 nm in diameter and ~70-nm high) that are embedded in the double-membraned nuclear envelope (NE). The NPC allows passive diffusion of ions and small molecules through an aqueous channel with a physical diameter of ~9 nm, and it mediates transport of proteins and ribonucleoprotein (RNP) particles through a gated channel with a functional diameter of up to 26 nm (Feldherr et al., 1984; Gerace, 1992). Nuclear protein import is selective, generally requires a nuclear localization signal (NLS), and is energy dependent.

The NE is a dynamic structure that undergoes dramatic changes during mitosis when it is reversibly disassembled (Gerace and Burke, 1988). Little is known about the disassembly of NPCs during mitosis and the biosynthesis and reassembly of NPCs after mitosis. Nevertheless, some advances in this field have been made by the use of *Xenopus* egg extracts which are able to assemble the NE in vitro (e.g., Finlay and Forbes, 1990; Dabauvalle and Scheer, 1991; Dabauvalle et al., 1991).

Structural studies have revealed the basic architecture of the NPC, biochemical characterization of NPC components is beginning to emerge, and a number of functional aspects of nucleocytoplasmic transport have been established. There remain, however, many questions and ambiguities that have to be resolved before NPC structure-function relationships will be understood at the molecular level. Here we review emerging structural and functional models, and propose future directions towards a complete molecular understanding of NPC structure and function. For background and further details the reader is referred to recent reviews (e.g., Garcia-Bustos et al., 1991; Goldfarb and Michaud, 1991; Nigg et al., 1991; Akey, 1992; Forbes, 1992; Gerace, 1992; Stewart, 1992).

Structural Organization of the Nuclear Pore Complex

The structure of the NPC has been extensively investigated by EM, and there is slowly but definitely emerging a consensus on its basic architectural framework (Unwin and Milligan, 1982; Akey, 1989; Reichelt et al., 1990; Jarnik and Aebi, 1991; Hinshaw et al., 1992; Akey and Radermacher, 1993). Accordingly, the NPC has been described as a tripartite assembly consisting of a spoke complex embracing a central channel complex which is sandwiched between a cytoplasmic and a nuclear ring. In support of this tripartite structure, it has been reported that chemical and mechanical treatments of whole nuclei or isolated NEs disassemble the NPC into distinct components, i.e., a channel-spoke complex, a cytoplasmic and a nuclear ring (Akey, 1989; Reichelt et al., 1990; Jarnik and Aebi, 1991; Hinshaw et al., 1992). When viewed perpendicular to the plane of the NE, the intact NPC, the spoke complex and both rings exhibit strong eightfold rotational symmetry.

Recently, a three-dimensional (3-D) reconstruction of negatively stained NPCs released from the NE by detergent treatment has been computed by the random conical tilt method (Hinshaw et al., 1992). As documented in Fig. 1 *a*, the basic framework of the NPC consists of two identical halves relative to the central plane of the NE and yields strong eightfold rotational symmetry in views perpendicular to the NE, hence, it has 822 symmetry. The map is dominated by eight multi-domain spokes where each of the eight spokes is built from four distinct morphological elements termed "annular," "column," "ring," and "lumenal" subunits (Fig. 1 *b*). As each spoke is made of two identical halves, there are two copies of each subunit per spoke or 16 copies per NPC. Via their annular subunits, the eight radial spokes are assembled around a ~40-nm-diam central pore. As indicated in Fig. 1 *c*, when the nuclear membranes are positioned in the 3-D map, eight "peripheral channels" (see also Fig. 1 *a*) with an average diameter of 10 nm are created at a radius of ~40 nm from the NPC center. As speculated by Hinshaw et al. (1992), these peripheral channels may represent sites for passive diffusion of ions and small molecules.

Most recently, Akey and Radermacher (1993) have computed 3-D maps from both membrane-associated (*mform*) and -demembranated (*dform*) NPCs by tomographic reconstruction from tilted views of frozen hydrated NPCs. Significant differences are observed between these two maps, due probably to loss of some NPC components and subsequently structural rearrangements within the spoke-ring assembly upon detergent treatment. At the 10-nm resolution level, comparison of these maps with the 3-D map of Hinshaw et al. (1992) reveals similar basic features for the domain structure of the spoke complex. However, there are two

1. *Abbreviations used in this paper*: EM, electron microscope/microscopy; HRSEM, high-resolution scanning EM; MDa, megadaltons = 10^6 daltons; NE, nuclear envelope; NL, nuclear lamina; NLS, nuclear localization signals; NPC, nuclear pore complex; RNP, ribonucleoprotein; SFM, scanning force microscopy; STEM, scanning transmission EM; TEM, transmission EM; WGA, wheat germ agglutinin.

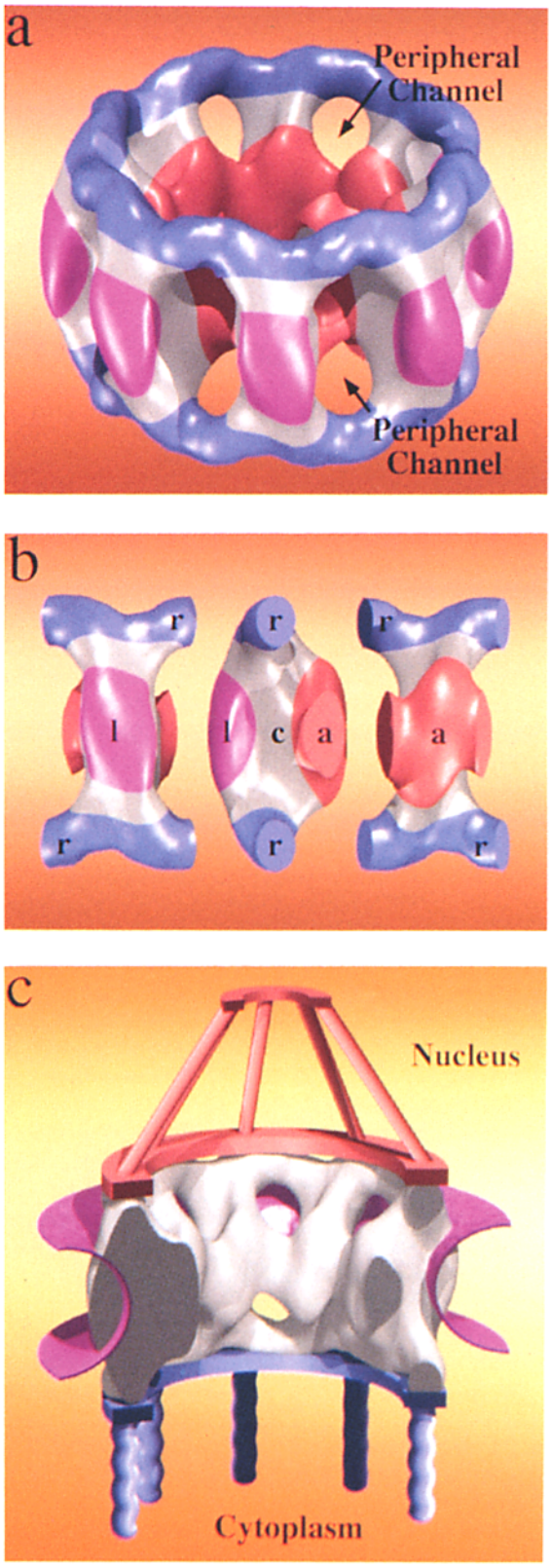

Figure 1. Surface renderings of the nuclear pore complex architecture. (*a*) An oblique view of the 3-D map representing the basic framework of the NPC which exhibits strong 822 symmetry and thus consists of two identical halves relative to the central plane of the nuclear envelope. This map has been adapted from Fig. 5 of Hinshaw et al. (1992). (*b*) One multi-domain spoke cut out from the basic framework of the NPC shown in *a*. Each half-spoke is built from four distinct morphological elements, i.e., the annular (*a*), column (*c*), ring (*r*), and lumenal (*l*) subunits. (*c*) Schematic diagram of an NPC embedded in the double-membraned nuclear envelope. Its major structural components include the basic framework (i.e., the spoke complex as shown in Fig. 1 *a*) and the peripheral cytoplasmic filaments and nuclear baskets which have been modeled based on data obtained by Ris (1991), Jarnik and Aebi (1991), and Goldberg and Allen (1992) (see also Fig. 2). In this diagram, we have also pictured an extra cytoplasmic and nuclear ring in addition to the two rings being integral parts of the basic framework via the ring subunits (see Fig. 1, *a* and *b*). Note that the central channel complex has been omitted in this NPC model.

main differences between these maps: (*a*) Instead of the eight *peripheral* channels depicted in the map of Hinshaw et al. (1992) (see above and Fig. 1), Akey and Radermacher's *dform* map yields eight *internal* channels located between the spokes and the central channel complex, which these authors suggest to represent sites for passive diffusion. (*b*) Whereas Hinshaw et al. (1992), due to its irreproducibility among NPCs, have omitted the central channel complex from their 3-D reconstruction, Akey and Radermacher's *dform* map includes an elaborate, barrel-like transporter (Akey and Radermacher, 1993). As discussed below, this in our opinion, remains a controversial NPC component which requires more systematic investigation before it can be decided how this relates to the actual transport machine.

Due primarily to its large size and unknown chemical composition (see below), the mass of the NPC has long been subject to speculation (e.g., Gerace and Burke, 1988). Recently, the mass of intact NPCs and of several distinct NPC components has been measured directly by quantitative scanning transmission EM (STEM) (Reichelt et al., 1990). Accordingly, the membrane-bound, intact NPC has a mass of 124 megadaltons (MDa), and the spoke complex, i.e., the basic framework of the NPC as shown in Fig. 1 *a*, 52 MDa. Since the spoke complex has 822 symmetry (see above), the mass of its asymmetric unit (i.e., one half-spoke) is ~3.3 MDa or on the order of a ribosome. Two types of rings were also measured in detergent-treated NPCs: heavy, 32-MDa, and light, 21-MDa rings. The 32-MDa rings were also observed as "footprints," i.e., cytoplasmic rings with one to several collapsed cytoplasmic filaments attached (see below), when nuclei were rolled back and forth on charged EM grids (Jarnik and Aebi, 1991). Frequently, the 21-MDa rings revealed mass in the center (see below), which due to its variation among rings was excluded from the mass measurements.

Although there is emerging a consensus on the basic framework of the NPC (see Fig. 1, *a* and *b*), some of the structural components of the NPC remain controversial. For example, as illustrated in Fig. 2 *a*, whereas in thin sections NPC-associated filaments were seen a long time ago, these were not revealed with other preparation techniques. Recently, these NPC-associated filamentous structures have been visualized independently by two methods: (*a*) high-resolution scanning EM (HRSEM) of critical point-dried/metal-sputtered isolated NEs (Ris, 1991; Goldberg and Allen, 1992), and (*b*) transmission EM (TEM) of quick-frozen/freeze-dried/rotary metal-shadowed isolated *Xenopus* oocyte NEs (Jarnik and Aebi, 1991). Both methods have yielded very similar structures: as illustrated in Fig. 2 *b*, the cytoplasmic face of the NPC is topped with a massive,

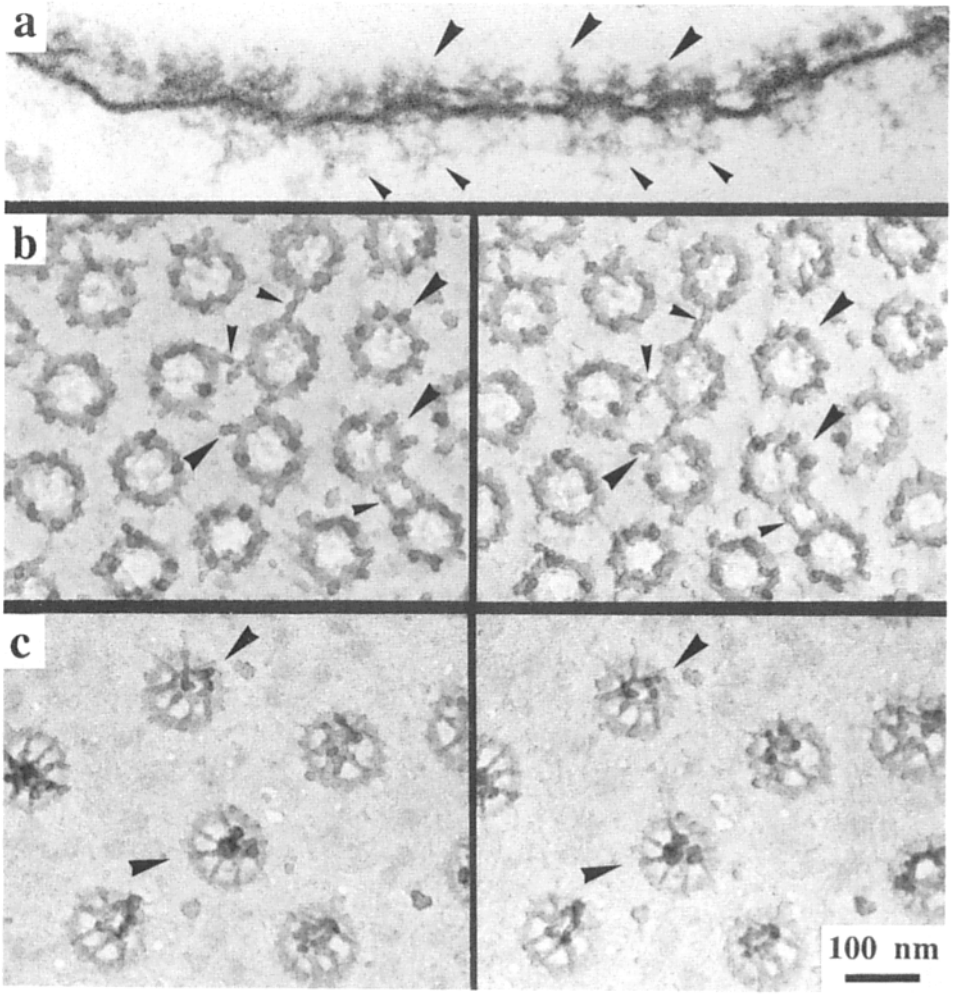

Figure 2. NPC-associated filaments revealed by (*a*) thin sectioning and (*b* and *c*) quick freezing/freeze drying/rotary metal shadowing. (*a*) *Xenopus* oocyte NE isolated in low-salt buffer, fixed with glutaraldehyde/tannic acid/OsO_4, and embedded in Epon (Jarnik and Aebi, 1991). Both cytoplasmic (*large arrowheads*) and nuclear (*small arrowheads*) filamentous structures emanate from the basic framework of the NPC. Stereo pairs of (*b*) the cytoplasmic, and (*c*) the nuclear faces of quick-frozen/freeze-dried/rotary metal-shadowed intact *Xenopus* oocyte NEs spread on an EM grid and chemically fixed with 2% glutaraldehyde/1% OsO_4 (Jarnik and Aebi, 1991). The large arrowheads in *b* point to "short cylinders" or "collapsed filaments" protruding from the cytoplasmic rings of the NPCs, whereas the small arrowheads in *b* mark "NPC connecting fibrils" which represent cytoplasmic filaments that have bent to the side and adhered to filaments of adjacent NPCs. The large arrowheads in *c* mark NPCs with relatively well-preserved nuclear baskets. Bar, 100 nm.

100–110-nm outer diameter ring, from which eight "short cylinders" or "collapsed filaments" protrude (Fig. 2 *b*, *large arrowheads*). The presence of these cytoplasmic filaments is best documented in situations where they have bent to the side and adhered to filaments of adjacent NPCs, thus appearing as "NPC connecting fibrils" (Fig. 2 *b*, *small arrowheads*). As shown in Fig. 2 *c*, the nuclear face of the NPC exhibits a more tenuous, 90–100-nm outer diameter ring, from which eight thin, 50–100-nm-long filaments emanate that are joined distally by a 30–50-nm diam terminal ring, thus forming a "basket" or "fishtrap." These cytoplasmic filaments and nuclear baskets make the NPC distinctly asymmetric relative to the plane of the NE. As documented in Fig. 3, *a* and *b*, this asymmetry can also been visualized by scanning force microscopy (SFM) of NE-bound NPCs kept in their native buffer environment. Consistent with the results obtained by EM of dehydrated, metal-coated NPCs (Jarnik and Aebi, 1991; Ris, 1991; Goldberg and Allen, 1992), the cytoplasmic face appears "donut-like" (Fig. 3 *a*), whereas the nuclear face yields an "inverted bowl-like" appearance (Fig. 3 *b*).

As to their possible functional role, the cytoplasmic filaments have been implicated in (*a*) docking material destined for trafficking across the NE to the NPC, and (*b*) delivering it to the central channel complex for active translocation (Gerace, 1992; Richardson et al., 1988). The nuclear baskets are "dynamic" structures in that they disassemble upon removal of divalent cations and reform upon their addition (Jarnik and Aebi, 1991), and therefore could be directly involved in the active transport of proteins or RNP particles through the NPC.

In the model presented in Fig. 1 *c*, we have added the cytoplasmic filaments and the nuclear basket to the basic framework of the NPC. In addition to the two rings defined by the ring subunits of the spokes (see Fig. 1, *a* and *b*), we have sandwiched the basic framework between an extra cytoplasmic and nuclear ring. As the 3-D maps of detergent-released NPCs (Hinshaw et al., 1992; Akey and Radermacher, 1993) do not reveal these extra cytoplasmic and nuclear rings, they represent most likely distinct structural components rather than being integral parts of the spoke complex. (*a*) Nuclear and cytoplasmic rings are released from the NPC after mechanical or chemical treatment (see above; Unwin and Milligan, 1982; Akey, 1989; Reichelt et al., 1990; Jarnik and Aebi, 1991; Hinshaw et al., 1992). (*b*) 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 (Fig. 2 *a*) reveal a tripartite structure of the NPC that cannot be solely explained by the basic framework of the NPC as presented in Fig. 1 *a*. (*c*) 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 ring have to be added to that of the basic framework (i.e., 52 MDa + 32 MDa + 21 MDa = 105 MDa; Reichelt et al., 1990).

Another controversial component of the NPC structure has been the "central plug" (Unwin and Milligan, 1982) or "central channel complex" (e.g., Gerace, 1992), which more recently has been implicated to represent the actual "transporter," modeled as a double-iris arrangement that can assume several distinct configurations as it actively transports molecules and particles through the NPC (Akey, 1990). The barrel-like model of the transporter as it has been reconstructed most recently by Akey and Radermacher (1993), however, does not explain in an obvious way such an iris-like gating mechanism. In contrast, the frequent lack of this morphological component has given rise to suggestions that the central plug may represent material actually being in transit rather than a constitutive component of the NPC (e.g., Jarnik and Aebi, 1991). In view of the new peripheral structural features described for the NPC (see above and Figs. 1 and 2), a substantial fraction of what in projection appears as the central plug may in fact represent the terminal ring of the nuclear basket (Jarnik and Aebi, 1991), or material in transit associated with it, which has been squashed into the pore upon embedding the NPC in a thin layer of negative stain (Unwin and Milligan, 1982; Reichelt et al., 1990; Jarnik and Aebi, 1991) or a thin ice film (Akey, 1989, 1990). The sensitivity of the basket with its terminal ring to mechanical or chemical manipulations on the one hand, and its possible implications in transport of material through the NPC on the other could explain both the existence and the variable morphology of what has been called the central plug or transporter. For example, when NEs before specimen preparation are treated with Cu-orthophenanthroline, an oxidizing agent causing S–S bridge formation, more than 95% of the NPCs reveal a strong central plug after negative staining. After quick freezing/freeze drying/rotary metal shadowing the same preparations yield (*a*) well preserved nuclear baskets with a massive terminal ring, and (*b*) a plug-like structure residing in the pore judged from stereo pairs of the cytoplasmic face of the NPC (our own unpublished data). As an alternative, the mass residing in the central pore may in fact represent a physical "plug" that is pulled or pushed out of the pore by an amount sufficient to allow a particular protein–receptor complex (see below) to pass through the pore, thereby acting as a gated channel.

The nature of the interaction of the NPC with the nuclear lamina (NL) is another unsolved question. Detergent treatment of NEs yields NPCs that are still attached to the NL (e.g., Aebi et al., 1986). More recently, Goldberg and Allen (1992), in addition to the NL, have described another fibrous lattice, termed the "NE lattice" or NEL, that is connected to the NPCs via their nuclear baskets. The chemical composition, specific interaction with the NPC, and function of the NEL remain to be established.

Chemical Composition of the Nuclear Pore Complex

In contrast to the relatively large amount of structural studies (see above), less is known about the chemical composition of the NPC. Only a small number of NPC proteins have been identified and described to date, probably accounting for less than 10% of the NPC mass (Gerace, 1992). The first NPC

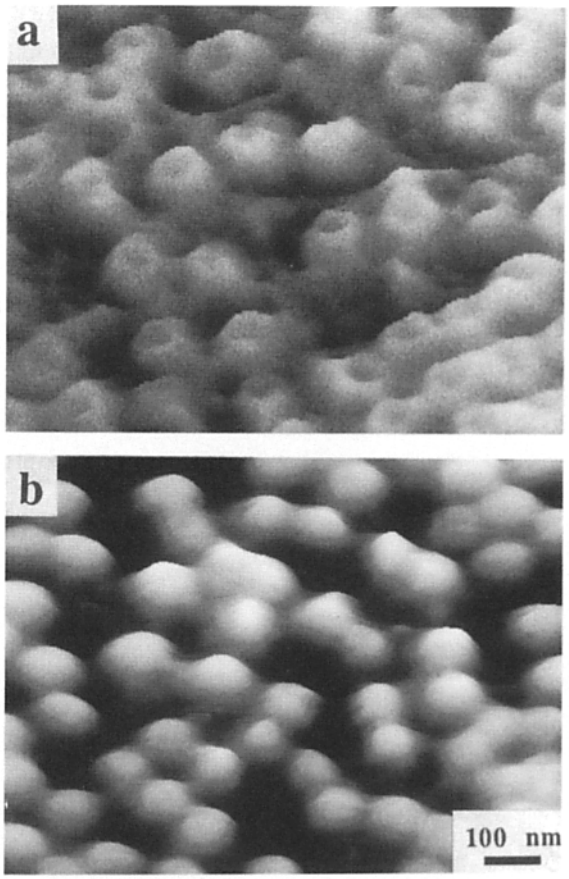

Figure 3. (*a* and *b*) Images of *Xenopus* oocyte NEs kept in their native buffer environment that were recorded with a SFM. Notice the asymmetric appearance of the cytoplasmic (*a*) and nuclear (*b*) faces: the NPCs appear "donut-like" on the cytoplasmic face, whereas they yield an "inverted bowl-like" appearance on the nuclear face. Bar, 100 nm.

polypeptide examined in some detail has been gp210, a transmembrane glycoprotein bearing N-linked (via Asp) high manose-type oligosaccharides (Gerace et al., 1982; Wozniak et al., 1989). This protein consists of a large (95% of its total mass) NH_2-terminal domain located 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 (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, it is tempting to speculate (Gerace, 1992) that the lumenal domain of gp210 forms part of the "knobs" (Jarnik and Aebi, 1991) or "lumenal" subunits (see Fig. 1) that have been shown to extend from the spokes radially into the lumen of the NE (Hinshaw et al., 1992; Akey and Radermacher, 1993). Thus gp210 may act as a membrane anchor for the NPC and/or play a topogenic role in membrane folding during nuclear pore formation (Greber et al., 1990; Jarnik and Aebi, 1991; Gerace, 1992; Hinshaw et al., 1992).

A group of mammalian NPC glycoproteins that are modified at up to 10–20 sites with O-linked N-acetylglu-

cosamine (GlcNac) have also been identified (Snow et al., 1987; Holt et al., 1987; Davis and Blobel, 1987). They have molecular weights ranging between 35 and 210 kD and are present in roughly 1–10 copies per NPC (Holt et al., 1987). These proteins appear to be involved in nucleocytoplasmic transport as it is inhibited by both mAbs to these glycoproteins (Featherstone et al., 1988) as well as the lectin wheat germ agglutinin (WGA) (Finlay et al., 1987; Dabauvalle et al., 1988). Furthermore, a complex consisting of at least three of these glycoproteins (p62, p58, and p54) is required for NPC function, as depletion of NPCs of this complex renders them unable to mediate protein import into the nucleus (Finlay et al., 1991). As illustrated in Fig. 4, EM localization studies of these O-linked glycoproteins in *Xenopus* oocyte NEs with colloidal gold-labeled WGA (Fig. 4, *a* and *b*) or antibodies to several of these glycoproteins (Fig. 4 *c*) have identified at least one of their sites near or at the terminal ring of the nuclear basket (see below).

The best studied member within this group of O-linked glycoproteins is the rat p62 (Davis and Blobel, 1986, 1987) and its homologues which have been cloned and sequenced in *Xenopus*, mouse, rat, and man (Starr et al., 1990; Carmo-Fonseca, et al., 1991; Cordes et al., 1991). Immunolocalization of p62 has revealed species-specific differences: a polyclonal mouse anti-p62 antibody labeled both the nucleoplasmic and cytoplasmic sides of mouse liver NEs but only the nucleoplasmic side of *Xenopus* oocyte NEs (Cordes et al., 1991). As documented in Fig. 4 *c*, using this antibody, we have more specifically localized p62 near or at the terminal ring of the nuclear baskets of *Xenopus* oocyte NEs. Analysis of the primary sequences of the various p62 homologues has indicated that the molecule is constructed from two distinct domains: (*a*) a highly conserved COOH-terminal domain revealing heptad repeats characteristic of an α-helical coiled–coil conformation; and (*b*) an NH_2-terminal domain containing 15–25 copies of a degenerate pentapeptide repeat (XFXFG) with a structure based on β-sheets. Whereas there is little direct sequence identity in the NH_2-terminal region, the repetitive motifs have been well preserved among homologues.

Several NPC proteins have been cloned and sequenced in yeast. Based on the occurrence of highly repeated motifs, these yeast NPC proteins can be divided into two groups. (*a*) NSP1 (Hurt, 1988), NUP1 (Davis and Fink, 1990), and NUP2 (Loeb et al., 1993), which contain several copies of a more or less degenerate pentapeptide motif (XFXFG) similar to those of the NH_2-terminal end of vertebrate p62. (*b*) NUP49, NUP100, and NUP146 (Wente et al., 1992), which contain a degenerate tetrapeptide motif (GLFG)—two of these proteins have been cloned and sequenced independently by Wimmer et al. (1992), and termed NSP49 and NSP116. These frequently observed repeated motifs in the different NPC proteins so far sequenced suggest that they may be assembled in a modular fashion which could account for their mutual interactions.

Another rat NPC protein, termed NUP153 or p180, has recently been cloned and sequenced (Sukegawa and Blobel, 1993). Based on its cDNA-deduced primary structure it has a calculated molecular mass of 152.8 kD, whereas by SDS-PAGE it runs with an apparent molecular mass of 180 kD. It shares a repetitive degenerate pentapeptide motif (XFXFG) with vertebrate p62, yeast NSP1 (Hurt, 1988) and

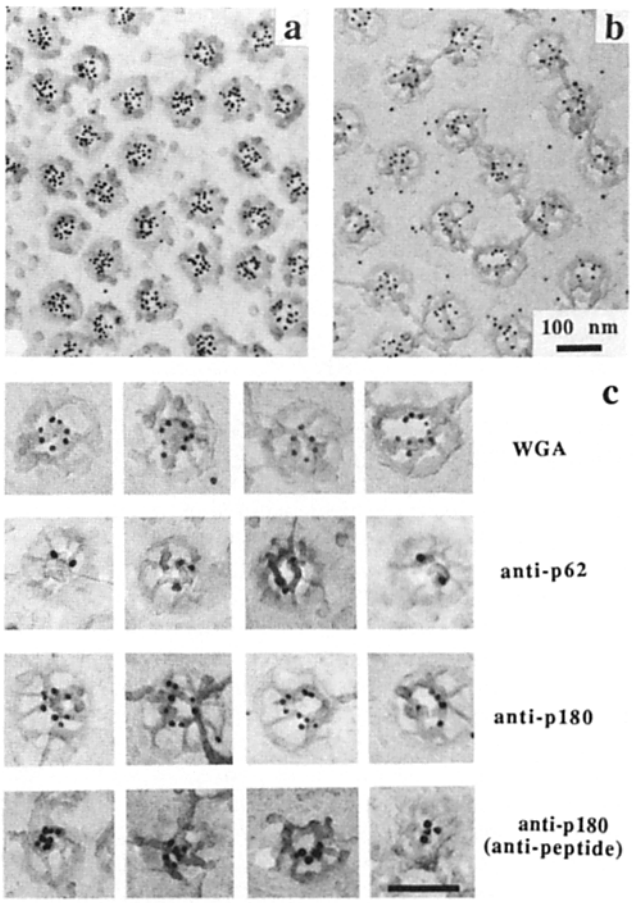

Figure 4. Immunolocalization of NPC proteins. (*a*) Cytoplasmic and (*b*) nuclear faces of quick-frozen/freeze-dried/rotary metal-shadowed intact spread *Xenopus* oocyte NEs labeled with WGA conjugated to ~8-nm colloidal gold. (*c*) Selected examples of nuclear faces of NPCs labeled with WGA, mouse anti-p62 antibody (a gift of Dr. G. Krohne), and human monoclonal anti-p180 and anti-peptide p180 antibodies (both provided by Dr. B. Burke) conjugated to ~8-nm colloidal gold. Notice that in all cases the gold tags are predominantly bound to the nuclear periphery of the NPCs near or at the terminal ring of the nuclear baskets. Bars, 100 nm.

NUP1 (Davis and Fink, 1990). Most striking, NUP153 contains four zinc finger motifs with two pairs of cysteines (Cys_2–Cys_2) which bind DNA. Immuno-EM localized NUP153 exclusively to the nucleoplasmic side of the NE (Sukegawa and Blobel, 1993). A human homologue of NUP153 has also been cloned and sequenced (Dr. B. Burke, personal communication). Its primary sequence is mostly conservative with the rat sequence and has also revealed a fourfold repeat of a Cys_2–Cys_2-type zinc finger motif. As documented in Fig. 4 *c*, using several monoclonal and polyclonal antibodies raised against the human homologue of NUP153, we have immunolocalized this novel protein at the terminal ring of the nuclear baskets (Panté, N., B. Burke, and U. Aebi, manuscript in preparation). Although many of these antibodies are polyspecific, reacting with more than one NPC polypeptide, they all label the terminal ring of the nuclear baskets (Fig. 4). Consistent with the localization of p180 and its content of zinc finger motifs, Zn^{2+} is one of the

most effective divalent cations to stabilize the nuclear baskets (Jarnik and Aebi, 1991).

In an attempt to more systematically identify NPC polypeptides, Radu et al. (1993) have recently described a procedure to isolate ~30 proteins from rat liver NEs that do not bind WGA. The most abundant of these proteins, a polypeptide with an estimated molecular mass of 140 kD by SDS-PAGE, was cloned and sequenced, and immunolocalized to the NPC. The deduced amino acid sequence revealed a protein of 155 kD (termed NUP155) that does not contain any obvious repetitive sequence motifs. It remains to be investigated how many more of these rat liver NE proteins that do not bind WGA are actually NPC proteins.

Functional Aspects of the Mediated Transport Through The Nuclear Pore Complex

Nuclear protein import is mediated by short amino acid sequences, called nuclear localization signals (NLSs), on the protein to be transported (Dingwall and Laskey, 1991; Garcia-Bustos et al., 1991). Most NLSs contain a high proportion of basic amino acid residues but there is no obvious consensus of them. The NLS of the Simian virus 40 (SV-40) large T antigen that contains a single contiguous stretch of basic amino acid residues (PK_{128}KKRKV; Garcia-Bustos et al., 1991) was considered to be a prototype NLS. However, the bipartite signal of *Xenopus* nucleoplasmin which contains two interdependent basic domains separated by 10 intervening "spacer" residues, may represent a more general type of NLS (Robbins et al., 1991). Since it is saturable and specific, nuclear import of carrier proteins tagged with synthetic NLS peptides has revealed protein import to be a receptor-mediated process (Garcia-Bustos et al., 1991). As a consequence, there has been considerable interest to identify the receptors that interact with NLSs to mediate nuclear import. Recently, two mammalian proteins, a 54- and a 56-kD polypeptide that bind the SV-40 large T antigen NLS have been isolated and demonstrated to exhibit the functional properties of NLS receptors (Adam and Gerace, 1991). Surprising, rather than being NPC-associated, these NLS receptors are predominantly cytosolic proteins which are also found in the nucleus (Adam et al., 1989), suggesting that they may function as "shuttling carriers" and thus be recycled for several rounds of transport (Adam et al., 1989; Adam and Gerace, 1991). In addition, two cytosolic factors, one required for binding of the protein–receptor complex to the NPC and the second one for translocation of the complex through the NPC, have been isolated (Newmeyer and Forbes, 1990; Moore and Blobel, 1992).

These recent advances on NLSs and NLS receptors suggest that the NPC-mediated transport pathway of proteins into the nucleus occurs by several distinct steps, some of them still being hypothetical (e.g., Goldfarb and Michaud, 1991; Nigg et al., 1991; Gerace et al., 1992; Stewart, 1992). (*a*) While in the cytoplasm, the protein to be imported is complexed to a cytosolic receptor via a specific NLS (Newmeyer and Forbes, 1988; Adam and Gerace, 1991). (*b*) Depending on additional cytosolic factors, this protein-receptor complex then docks to an NPC by specific binding to some "peripheral" NPC structure such as the cytoplasmic ring or the cytoplasmic filaments (see Fig. 1 *c*) (Richardson et al., 1988; Sterne-Marr et al., 1992). (*c*) From this peripheral docking site the protein–receptor complex is next delivered to the central channel complex which harbors the actual transport machine. (*d*) Active translocation of the protein-receptor complex through the central channel complex occurs after channel gating to accommodate the particular size and shape of the protein–receptor complex. (*e*) After release into the nucleus, the protein–receptor complex dissociates, and the receptor may be recycled for further rounds of transport (Adam et al., 1989; Adam and Gerace, 1991). In this multi-step model for protein import several issues remain elusive. For example, the site(s) and mechanism(s) of ATP utilization: as it occurs at 0°C and after ATP depletion, docking of the protein–receptor complex to the cytoplasmic NPC surface is not energy consuming (Newmeyer and Forbes, 1988; Richardson et al., 1988). One possible role for ATP may involve delivery of the protein–receptor complex from the peripheral docking site to the central channel complex, and furthermore, ATP is likely to be involved in channel gating and translocation. Among many possibilities, ATP may be used by a mechanochemical enzyme system, and/or a coupled system involving protein kinases that phosphorylate distinct NPC components, and phosphatases that undo this modification (Nigg et al., 1991; Gerace et al., 1992). Another issue which remains elusive is the nature and mechanism of the gated channel. One possibility is a plug-type mechanism where the plug can be pulled or pushed out of the pore by just the amount required for a particular protein–receptor complex to move through the pore.

Similar to protein import, export of RNA through the NPC is also a signal-dependent, receptor-mediated process that requires ATP. Although both import and export use the same translocation machinery and are inhibited by WGA and antibodies to NPC proteins (Featherstone et al., 1988), the targeting mechanisms seem to be different. The import of small nuclear ribonucleoprotein (U2 snRNP) particles, where both protein and RNA motifs appear to have a targeting role, has been studied in some detail (Michaud and Goldfarb, 1992). In contrast, for the export of RNA some studies have indicated that it depends upon its association with proteins which may have a NLS similar to that used in nuclear protein import (Guddat et al., 1990). Since monomethylate cap structures facilitate export of RNA, it has also been suggested that the signal resides on the primary structure of the RNA (Hamm and Mattaj, 1990). Moreover, as yet the nuclear site(s) where these signals exert their effect is unclear. The RNA-associated proteins may play a "shuttling" role similar to NLS receptors for protein import (see above). Apparently, RNA export is a polar process: e.g., transport of large RNP particles such as the Balbiani ring granules of *Chironomus* requires the 5′ end of the RNA to pass through the NPC first (Mehlin et al., 1992).

Conclusions and Future Prospects

Considering the size and complexity of the NPC, significant progress has been made over the past few years in exploring its basic architectural framework and establishing the steps involved in nuclear import of proteins through the NPC. Signals and receptors that mediate import of proteins have been identified and characterized. However, only limited information has been gathered about the chemical composition of the NPC. Nevertheless, the first NPC polypeptides have just been immunolocalized in three dimensions (e.g., Fig. 4). An important next step will be to more systematically define

(both chemically and structurally) the different molecular components of the NPC, including the central channel complex, and to unveil the 3-D *molecular* architecture of the NPC both of its basic framework and its peripheral components such as the cytoplasmic filaments and nuclear baskets. Another important step will be to decipher the functional roles of these distinct structural components: as yet, the structural models of the NPC are too "premature" to directly correlate them with nucleocytoplasmic transport. Obviously, all this structural information at the molecular level will be key to eventually arrive at the molecular mechanisms underlying nucleocytoplasmic transport. Evidence for the existence of at least one other signaling pathway has been described for the import of RNP particles (Michaud and Goldfarb, 1992). It is conceivable that additional nuclear import–export pathways will be detected in the near future, some of which might be specific to certain cell types or differentiation stages. Presumably, all these signaling pathways use a common transport machinery of the NPC which resides in the central channel complex, so it will be important to define and characterize this molecular machine in greater detail.

Thus, to eventually arrive at a complete molecular understanding of NPC structure and function, we have to pool all available forces and methodologies commonly used in cell, molecular and structural biology, and complement these with hard-core biochemistry and state-of-the-art biophysical techniques. One great promise holds the use of scanning force microscopy both to image (see Fig. 3), and to mechanically and chemically manipulate NPCs in their native environment, thus providing us with the exciting possibility to directly correlate NPC structure with function.

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