

MINIREVIEW

Toward the Molecular Details of the Nuclear Pore Complex

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INTRODUCTION

The nuclear pore complex (NPC) is a ~120-megadalton (MDa) macromolecular assembly embedded in the double-membraned nuclear envelope (NE) that mediates bidirectional molecular trafficking between the cytoplasm and the nucleus of interphase cells. The structure of the NPC has been extensively investigated by different electron microscopy (EM) specimen preparation methods and imaging techniques (reviewed by Panté and Aebi, 1993, 1994), including scanning force microscopy (SFM) in physiological buffer environment (Panté and Aebi, 1993; Goldie *et al.*, 1994; Oberleithner *et al.*, 1994). These structural studies have revealed that the NPC is composed of a 52-MDa basic framework made of eight multidomain “spokes” (Hinshaw *et al.*, 1992; Akey and Radermacher, 1993) embracing a “central pore” which is sometimes “plugged” with a “central channel complex” (also called “central plug” or “transporter”). The spoke complex is sandwiched between a nuclear and a cytoplasmic ring to both of which distinct filamentous structures are attached: the cytoplasmic ring is decorated with eight short, kinky filaments, 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 (Jarnik and Aebi, 1991; Ris, 1991; Goldberg and Allen, 1992). In contrast to the relatively large number of structural studies, less is known about the chemical composition and molecular architecture of the NPC. Based on its molecular mass of about 120 MDa (Reichelt *et al.*, 1990), it is believed that the

NPC is composed of multiple copies (i.e., 8 or 16) of on the order of 100 different proteins. For several years, gp210 and p62 have been the only two well-characterized NPC polypeptides. However, over the last 2 years about two dozen NPC proteins have been identified, and many of them have now been cloned and sequenced. Moreover, the combination of well-characterized antibodies with different EM specimen preparation methods has allowed localization of several of these proteins to distinct structural components of the NPC. Thus the *molecular* architecture of the NPC is now definitely on its way to being elucidated. Here we review the presently identified NPC proteins and the recent progress made toward their localization within distinct structural components of the NPC.

NPC PROTEINS

As summarized in Table I, three major groups of NPC proteins have so far been identified and characterized. These are: (i) integral membrane proteins which are associated with the nuclear membrane and therefore are not extracted even after treatment with alkaline pH or chaotropic agents (Gerace *et al.*, 1982); (ii) peripheral membrane proteins which are not associated with or anchored in the nuclear membrane—members of this group have been called “nucleoporins” (Davis and Blobel, 1986); and (iii) yeast NPC proteins whose exact relationship to the vertebrate NPC proteins is not yet known. According to a recently proposed nomenclature, the integral membrane proteins of the NPC have been denoted POMx (for pore membrane protein; Hallberg *et al.*, 1993) and the peripheral membrane proteins of the NPC

TABLE I
Classification of NPC Proteins

Type	Name	Molecular mass ^a (kDa)	Characteristics of primary and predicted secondary structure	Other properties and possible functions	Location	References
Integral membrane proteins of the NPC	gp210	210	21-Residue-long transmembrane domain between a 58-residue-long C-domain ^b and a 1783-residue-long N-domain ^c	Bears N-linked (via Asp) high mannose oligosaccharides Reacts with ConA Antibodies against luminal domain inhibit NPC function Possibly anchors the NPC to the NE	Most of its mass (N-domain) resides in the lumen of the NE	Gerace <i>et al.</i> (1982); Wozniak <i>et al.</i> (1989); Greber <i>et al.</i> (1990)
	POM121	121	44-Residue-long transmembrane domain between a 28-residue-long N-domain and a 1127-residue-long C-domain Repetitive XFXFG motifs at C-terminal third	Binds WGA Possibly anchors the NPC to the pore membrane	Most of its mass (C-domain) resides in the NPC proper	Hallberg <i>et al.</i> (1993)
	POM152 (yeast)	152	20-Residue-long transmembrane domain between a 175-residue-long N-domain and a 1142-residue-long C-domain	Reacts with ConA Possibly anchors the NPC to the pore membrane	Most of its mass (C-domain) resides in the lumen of the NE	Wozniak <i>et al.</i> (1994)
Peripheral membrane proteins of the NPC	p62	62 ^d	α -Helical coiled-coil C-domain Repetitive XFXFG motifs at N-domain	Binds WGA Exists as a complex (p62-p58-p54) Required for NPC function	Central plug or channel complex ^e	Starr <i>et al.</i> (1990); Carmo-Fonseca <i>et al.</i> (1991); Cordes <i>et al.</i> (1991); Finlay <i>et al.</i> (1991)
O-Linked glyco-proteins	NUP153	153	Four zinc finger motifs Repetitive XFXFG motifs at C-terminal 2/3	Binds WGA Exists as a homooligomer of ≥ 1 MDa Binds DNA <i>in vitro</i>	Terminal ring of the nuclear basket	Sukegawa and Blobel (1993); McMorro <i>et al.</i> (1994); Cordes <i>et al.</i> (1994)
	CAN/NUP214/p250	214	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	Cytoplasmic filaments	Kraemer <i>et al.</i> (1994); Panté <i>et al.</i> (1994)
Non-O-linked proteins	NUP107	107	Leucine zipper motif	Does not bind WGA	Unknown	Radu <i>et al.</i> (1994)
	NUP155	155	Nonrepetitive motifs	Does not bind WGA	Unknown	Radu <i>et al.</i> (1993)
	NUP180	180 ^f	Unpublished	Does not bind WGA	Cytoplasmic ring or filaments	Wilken <i>et al.</i> (1993)
	Tpr/p265	265	\sim 1600-Residue-long α -helical coiled-coil region Acidic C-domain	Very prone to proteolysis with a major proteolytic product of \sim 180 kDa	Cytoplasmic filaments	Byrd <i>et al.</i> (1994)
Yeast NPC proteins	POM152 (see above)					
XFXFG family	NSP1	87	Repetitive XFXFG motifs	Essential for cell growth	Unknown	Hurt (1988)
	NUP1	114	Repetitive XFXFG motifs	Essential for cell growth	Unknown	Davis and Fink (1990)
	NUP2	95	Repetitive XFXFG motifs	Not required for cell growth Forms a complex with NUP1	Unknown	Loeb <i>et al.</i> (1993); Belanger <i>et al.</i> (1994)
	NUP/NSP49	49	Repetitive GLFG motifs at N-domain	Essential for cell growth	Unknown	Wente <i>et al.</i> (1992); Wimmer <i>et al.</i> (1992)
	NUP100	100	Repetitive GLFG motifs at N-domain	Not required for cell growth	Unknown	Wente <i>et al.</i> (1992); Fabre <i>et al.</i> (1994)

TABLE I—Continued

Type	Name	Molecular mass ^a (kDa)	Characteristics of primary and predicted secondary structure	Other properties and possible functions	Location	References
GLFG family	NUP/NSP116	116	Repetitive GLFG motifs at N-domain RNA-binding motifs	Deletions in the <i>nup116</i> gene yield sealed NPCs	Unknown	Wente <i>et al.</i> (1992); Wimmer <i>et al.</i> (1992) Wente and Blobel (1993); Fabre <i>et al.</i> (1994)
	NUP145	145	Repetitive GLFG motifs at N-domain RNA-binding motifs	Deletions/disruptions in the <i>nup145</i> gene yield clusters of sealed NPCs	Unknown	Fabre <i>et al.</i> (1994); Wente and Blobel (1994)
	NIC96	96	Nonrepetitive motifs	Forms a complex with NSP1 and NUP49	Unknown	Grandi <i>et al.</i> (1993)

^a Calculated from the amino acid sequence.

^b COOH-terminal domain.

^c NH₂-terminal domain.

^d Depending on species.

^e Guan *et al.*, manuscript in preparation.

^f Based on SDS-PAGE.

or nucleoporins NUPx (Wente *et al.*, 1992), where x indicates the predicted molecular mass in kilodaltons based on their amino acid sequence. Within the NPC distinct sets of these proteins may interact with each other, thus forming subcomplexes which may be isolated as such. Recently, at least three distinct subcomplexes in vertebrate NPCs and two in yeast NPCs have been identified (see below). The functional role of such subcomplexes and/or of individual NPC polypeptides is still speculative. The proposed role of some of these proteins is based on their identification with distinct structural components of the NPC. For example, the cytoplasmic filaments have been proposed to represent "docking sites" for proteins to be imported into the nucleus. Following is a description of the presently identified members of these three groups of NPC proteins, the localization of some of their epitopes as recognized by specific antibodies in the 3-D structure of the NPC, and the few so far characterized NPC subcomplexes.

INTEGRAL MEMBRANE PROTEINS OF THE NPC

The first NPC protein identified in rat liver NEs was gp210, a transmembrane 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 in Fig. 1, the topology of gp210 as determined by the use of site-specific antibodies and proteolytic digestions (Greber *et al.*, 1990) has revealed that gp210 consists of a large (~95% of its total mass) NH₂-terminal domain residing in the lumen of the NE, a single, 21-residue-long transmembrane segment, and a short, 58-residue-long COOH-terminal domain associated with the NPC. The transmembrane segment of gp210 has been shown

to be sufficient for targeting this protein to the nuclear membrane (Wozniak and Blobel, 1992). Based on the topology of gp210 (see Fig. 1), there have been speculations that the luminal domain of this protein forms part of the "knobs" or "luminal" 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). For its possible functional task, gp210 has been proposed to act as a membrane anchor for the NPC and/or to have a topogenic role in membrane folding during nuclear pore formation (Greber *et al.*, 1990; Jarnik and Aebi, 1991; Gerace, 1992; Hinshaw *et al.*, 1992). Somewhat unexpectedly, an antibody directed against the luminal domain of gp210 inhibits both passive diffusion of small molecules and mediated nuclear import of proteins (Greber and Gerace, 1992).

A second integral membrane protein of the NPC, POM121, has recently been identified in rat NEs and cloned and sequenced (Hallberg *et al.*, 1993). Similar to some of the peripheral membrane glycoproteins of the NPC (see below and Table I), POM121 binds wheat germ agglutinin (WGA), a lectin that recognizes O-linked *N*-acetylglucosamine (GlcNAc) residues. Moreover, the amino acid sequence of POM121 has revealed the presence of a repetitive pentapeptide motif XFXFG (where X indicates any amino acid) which is also present in the members of the O-linked NPC glycoprotein family as well as some yeast NPC proteins (see below and Table I). As illustrated in Fig. 1, the primary sequence of POM121 has revealed a 44-residue-long transmembrane domain sandwiched between a short NH₂-terminal tail (28 residues long) and a long COOH-terminal domain. Since the latter contains the repetitive XFXFG motifs, it has been pre-

dicted that the small NH₂-terminal tail resides in the lumen of the NE (see Fig. 1). Thus, in contrast to gp210, most of the mass of POM121 is predicted to reside within the NPC proper. While antibodies directed against POM121 clearly labeled the NPC, the exact location of this protein within the NPC remains to be determined. Very much like gp210, POM121 has been proposed to function as a membrane anchor of the NPC.

Using the isolation procedure of yeast NPCs (Rout and Blobel, 1993) in combination with the biochemical approach used to identify gp210 (Gerace *et al.*, 1982), an integral membrane protein of yeast NPCs, POM152, has recently been identified (Wozniak *et al.*, 1994). As does 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 region adjacent to the NH₂-terminal side of the transmembrane segment being similar to 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. Since the COOH-terminal domain (residues 196–1337) of POM152 contains three putative sites for N-linked glycosylation with at least one of them being glycosylated, in analogy to gp210 (see above), this domain has been proposed to reside in the lumen of the NE (see Fig. 1). However, the exact topology of this protein remains to be established.

PERIPHERAL MEMBRANE PROTEINS OF THE NPC

O-Linked Glycoproteins

A group of at least eight NPC glycoproteins that are modified at up to 10–20 sites with *O*-linked *N*-acetylglucosamine, and therefore bind WGA, has originally been identified in rat liver NEs (Snow *et al.*, 1987; Holt *et al.*, 1987; Davis and Blobel, 1987). These proteins, estimated by SDS-PAGE, have molecular masses of 45, 54, 58, 62, 100, 145, 180, and 210 kDa, and they are present in roughly 1–10 copies per NPC (Holt *et al.*, 1987). Since both monoclonal antibodies to these proteins (Dabauvalle *et al.*, 1988a; Featherstone *et al.*, 1988) and WGA (Finlay *et al.*, 1987; Dabauvalle *et al.*, 1988b) inhibit import of nuclear proteins, it has been speculated that these *O*-linked glycoproteins might be involved in mediated nuclear import. As summarized in Table I, the 62- and 180-kDa *O*-linked glycoproteins have now been cloned and sequenced and termed p62 (Starr *et al.*, 1990; Carmo-Fonseca *et al.*, 1991; Cordes *et al.*, 1991) and NUP153 (Sukegawa and Blobel, 1993; McMorro *et al.*, 1994). The ~210-kDa glycoprotein originally identified by Snow *et al.*, (1987) has recently been demonstrated to be a homologue of human CAN (Kraemer *et al.*, 1994), a putative onco-

gene product associated with myeloid leukemogenesis (Von Lindern *et al.*, 1992). Hence, this protein has been termed CAN/NUP214. Consistent with being peripheral membrane proteins, two of these *O*-linked glycoproteins—NUP153 and CAN/NUP214—have now been localized to distinct peripheral components of the NPC (see below).

As illustrated in Fig. 2a, the cDNA-deduced amino acid sequences of the three *O*-linked NPC glycoproteins thus far cloned and sequenced revealed the presence of several copies of a more or less degenerate pentapeptide (XFXFG) motif which is considered to be a diagnostic feature for the members of the *O*-linked NPC glycoprotein family and may account for their mutual interactions within NPC subcomplexes (see below). This repeated motif is clustered in each protein: within the NH₂-terminal half of p62 and within the COOH-terminal domain of NUP153 and CAN/NUP214. As indicated in Fig. 2a, in addition to the NH₂-terminal domain containing multiple copies of the XFXFG motif, the COOH-terminal half of p62 contains heptad repeats reminiscent of two-stranded α -helical coiled-coil conformations. Recently, p62 has been expressed in *Escherichia coli* and the recombinant protein visualized in the EM after glycerol spraying/rotary metal shadowing (Buss *et al.*, 1994). Accordingly, recombinant p62 appears as a ~35-nm-long rod-shaped molecule with a slight protuberance at the NH₂-terminal end, thus confirming the α -helical coiled-coil conformation of the COOH-terminal domain of p62. In addition, circular dichroism of recombinant p62 has indicated that its repetitive NH₂-terminal domain may have a cross- β conformation (Buss *et al.*, 1994). NUP153 is unique among the *O*-linked NPC glycoproteins identified and characterized to date in that its primary sequence harbors four zinc finger motifs, each containing two pairs of cysteine residues (Cys₂-Cys₂) (Sukegawa and Blobel, 1993; McMorro *et al.*, 1994). Since 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 NPCs (Sukegawa and Blobel, 1993). Another possibility is that the zinc finger motifs of NUP153 might bind RNA *in vivo*, thus facilitating nuclear export of RNAs. In addition to the XFXFG repetitive motif, CAN/NUP214 contains multiple copies of the tripeptide motif FGQ that is also present in two yeast NPC proteins, i.e., NUP100 and NUP/NSP116 (see below; Wentz *et al.*, 1992; Wimmer *et al.*, 1992), and of the degenerate tetrapeptide motif SVFG and the tripeptide motif FGG that have

so far not been found in other NPC proteins. CAN/NUP214 also contains a leucine zipper motif that may function as a protein-protein dimerization domain (Von Lindern *et al.*, 1992).

Non-O-Linked Proteins

A group of at least 30 proteins that do not contain GlcNac have recently been identified in rat liver NEs (Radu *et al.*, 1993). This group of proteins has been separated from the O-linked NPC glycoproteins by WGA-Sepharose affinity chromatography and further purified on an SDS-hydroxylapatite column (Radu *et al.*, 1993). Two of these proteins—NUP155 (Radu *et al.*, 1993) and NUP107 (Radu *et al.*, 1994)—have been cloned and sequenced, and their deduced amino acid sequences do not reveal any of the repetitive sequence motifs (i.e., the XFXFG motif) which seem to be a diagnostic feature for the O-linked NPC glycoproteins (see Table I and Fig. 2a). As illustrated schematically in Fig. 2b, like CAN/NUP214 (see above), NUP107 contains a leucine zipper motif at its COOH-terminal end which has been suggested to induce dimerization with a second leucine zipper-containing polypeptide (Radu *et al.*, 1994). Anti-peptide antibodies against both NUP155 (Radu *et al.*, 1993) and NUP107 (Radu *et al.*, 1994) were raised and used to label several types of cultured cells by immunofluorescence microscopy and immuno-EM. Both antibodies labeled the NPCs of these cells, thus documenting that NUP155 and NUP107 are *bona fide* NPC proteins. Unfortunately, the ultrastructure of these NPCs has not yet been revealed; thereby, localization of these two proteins to distinct NPC components remains to be determined.

Using autoimmune antibodies, two new non-O-linked NPC proteins have recently been identified: (i) NUP180, a 180-kDa polypeptide identified in *Xenopus* oocyte NEs using a serum from a patient with overlap connective tissue disease (Wilken *et al.*, 1993); and (ii) p265, a 265-kDa protein identified in rat liver NEs using both monoclonal and autoimmune antibodies (Byrd *et al.*, 1994). The latter protein has been demonstrated to represent the rat homologue of human Tpr (translocated promoter region), a ~265-kDa protein whose NH₂-terminal domain appears in oncogenic fusions with the *met*, *trk*, and *raf* proto-oncogenes (Mitchell and Cooper, 1992). 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 (see Table I). As illustrated in Fig. 2b, based on its deduced amino acid sequence Tpr contains an over 1600-residue-long region predicted to form an α -helical coiled-coil via its heptad repeats (Mitchell and Cooper, 1992). It has further been shown that p265 is very prone to

proteolysis with a major ~175-kDa proteolytic product (Byrd *et al.*, 1994), suggesting that this product might be identical to NUP180, the 180-kDa non-O-linked NPC polypeptide identified by Wilken *et al.*, (1993). However, recent cloning and sequencing of NUP180 has not revealed any similarity with the primary sequence of Tpr/p265 (M.-C. Dabauvalle, personal communication). As we will discuss below, both NUP180 and Tpr/p265 have been localized to the cytoplasmic periphery of the NPC (see Figs. 4 and 5).

Yeast NPC Proteins

The use of antibodies against vertebrate NPC proteins in concert with the design of genetic screens has allowed identification of at least nine yeast NPC proteins (see Table I; reviewed by Fabre and Hurt, 1994; Rout and Wentz, 1994). The number of these is expected to rapidly grow, now that a procedure to bulk-isolate NPCs from yeast has been developed (Rout and Blobel, 1993). As mentioned above, one of these nine identified yeast NPC proteins, POM152, is an integral membrane protein (see Fig. 1 and Table I). The rest of them can be classified into three groups based on the occurrence of highly repetitive sequence motifs (see Table I and Fig. 3). First is the XFXFG family which contains several copies of a more or less degenerate pentapeptide motif (XFXFG) clustered in the central part of each protein (see Fig. 3a). While this XFXFG motif is a diagnostic feature for the vertebrate O-linked NPC glycoproteins (see above and Fig. 2a), it is not clear whether any of these yeast NPC proteins are in fact glycosylated. Members of this XFXFG family include NSP1 (Hurt, 1988), NUP1 (Davis and Fink, 1990), and NUP2 (Loeb *et al.*, 1993). A second group represents the GLFG family which contains multiple copies of a degenerate tetrapeptide motif (GLFG) within the NH₂-terminal domain (see Fig. 3b). Members of this group include NUP/NSP49, NUP100, NUP/NSP116, and NUP145 (Wentz *et al.*, 1992; Wimmer *et al.*, 1992; Fabre *et al.*, 1994; Wentz and Blobel, 1994). Finally, the third group is defined by the yeast nucleoporin interacting component NIC96 (see Fig. 3c) that has not revealed any repetitive sequence motifs and forms a complex with NSP1 and NUP49 (Grandi *et al.*, 1993).

Although most of the vertebrate and yeast NPC proteins contain short repetitive sequence motifs, in particular the XFXFG motif, no obvious relationship has yet been depicted between these vertebrate and yeast proteins. The only exception is NSP1 that has been considered the yeast homologue of vertebrate p62: both proteins have the same domain structure (see Figs. 2a and 3a) with 50% similarity in their COOH-terminal domains (Carmo-Fonseca *et al.*, 1991; Fabre and Hurt, 1994).

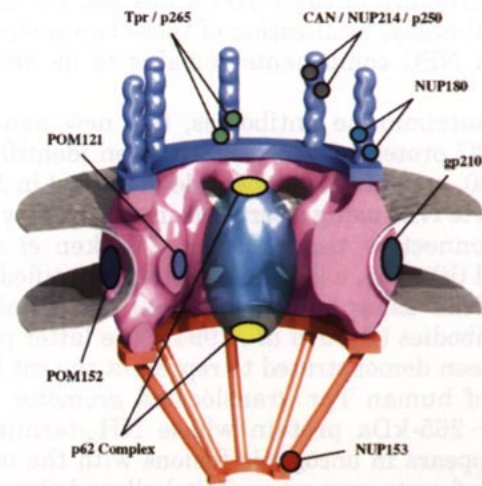
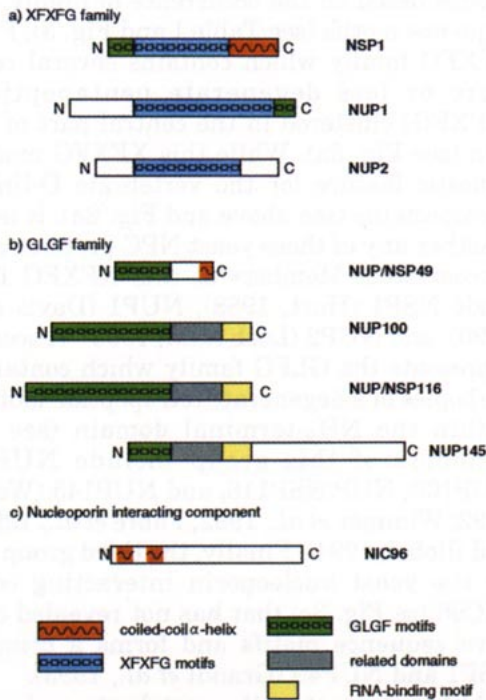
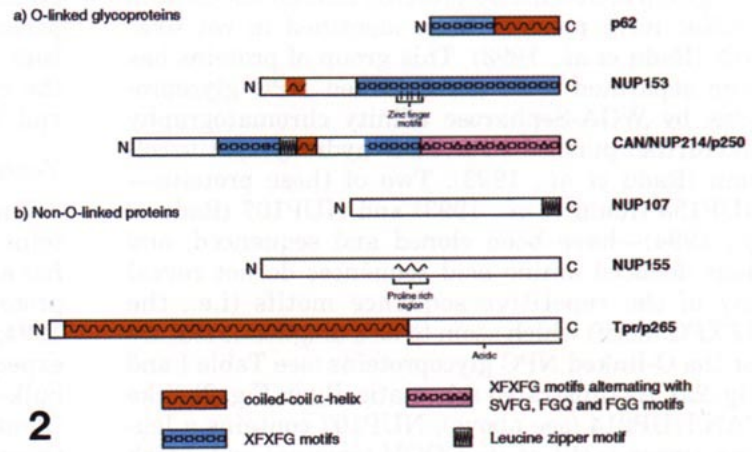
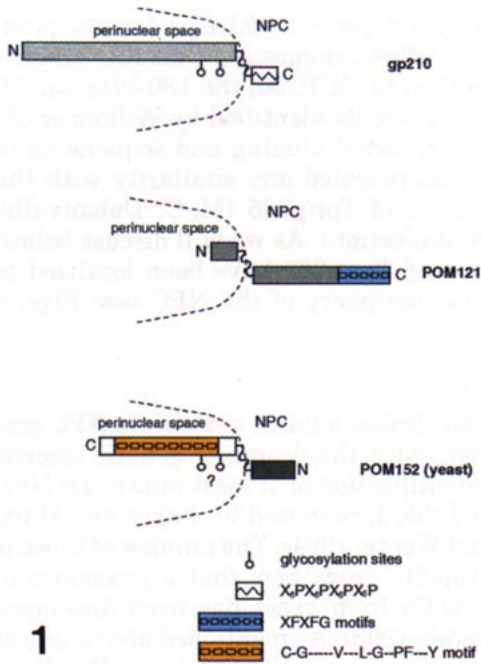


FIG. 1-3, 5. FIG. 1. Schematic diagram of the domain architecture and membrane topology of the cloned and sequenced integral membrane proteins of the NPC deduced from their amino acid sequences. Three transmembrane NPC glycoproteins, gp210, POM121, and the yeast POM152, have thus far been cloned and sequenced. They all reveal a distinct stretch of hydrophobic residues that is predicted to be a transmembrane segment traversing the pore membrane. gp210 contains a large NH₂-terminal domain residing in the lumen of the NE and a small COOH-terminal tail associated with the NPC. In contrast, POM121 consists of a short NH₂-terminal tail residing in the lumen of the NE and a long COOH-terminal tail—with a number of repetitive pentapeptide motifs XFXFG at its COOH-terminal end—associated with the NPC. Very much like gp210, most of the mass of yeast POM152 is predicted to reside in the lumen of the NE. In addition, the amino acid sequence of POM152 contains eight repetitive segments, each 24 residues long, with the consensus sequence C-G---V---L-G--PF---Y. The N-linked glycosylated residues of gp210 occur in the luminal domain close to the nuclear membrane (Greber *et al.*, 1990). By analogy, the N-linked glycosylated sites of POM152 are speculated to be located in the luminal domain too (Wozniak *et*

As illustrated in Fig. 3b, three members of the GLFG family, NUP100, NUP/NSP116, and NUP145, contain highly homologous sequence regions including an RNA-binding motif (Fabre *et al.*, 1994). Fragments of NUP/NSP116 and NUP145 including the RNA-binding motif have been expressed as fusion proteins in *E. coli*, and these have been demonstrated to bind RNA *in vitro* (Fabre *et al.*, 1994). Based on these results, it has been suggested that NUP/NSP116 and NUP145 may play a role in RNA recognition and/or transport through the NPC (Fabre and Hurt, 1994; Fabre *et al.*, 1994).

Using mutant strains, the genes for several yeast NPC proteins have been shown to be essential for cell growth (reviewed by Fabre and Hurt, 1994). Some of these yeast mutants have been examined in the EM to determine whether they perturb the structure of the NPC and/or the NE (Wente and Blobel, 1993, 1994). Accordingly, a membrane seal was formed over the cytoplasmic face of the NUP/NSP116-deficient NPCs which did not block nuclear export but caused the export substrate to accumulate within the cytoplasmic membrane herniations covering the NPCs (Wente and Blobel, 1993). Similarly, deletion/disruption of the NH₂-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 results, it has been proposed that NUP/NSP116 and NUP145 are possibly involved in establishing specific NPC-NE interactions and/or mediating NPC biogenesis (Wente and Blobel, 1993, 1994).

TOWARD THE MOLECULAR ARCHITECTURE OF THE NPC

As documented in Fig. 4, epitopes of five different NPC proteins have recently been localized within the 3-D NPC architecture. Three of these proteins—p62, NUP153, and CAN/p250—are members of the O-linked glycoprotein family (see Fig. 2a and Table I). Due to the relatively strong cross-reactivity of some of the antibodies against NPC proteins, the localization of p62 has remained ambiguous (Cordes *et al.*, 1991; Panté and Aebi, 1993). To resolve this ambiguity, we have recently produced a monoclonal antibody, RL31, which reacts specifically with rat p62 (Guan *et al.*, manuscript in preparation). As illustrated in Fig. 4a, RL31 labels both the nuclear and cytoplasmic periphery of the central plug or channel complex of rat liver NPCs, albeit the labeling at the nuclear periphery is more frequent. This localization is consistent with the supposed involvement of the p62 complex for nuclear import of proteins (Finlay *et al.*, 1991).

Using a polyclonal antibody raised against a fu-

al., 1994). For more information about these proteins, see Table I and references therein. FIG. 2. Schematic diagram of the domain architecture of the cloned and sequenced peripheral membrane proteins of the NPC deduced from their amino acid sequences. Depending on the content of O-linked *N*-acetylglucosamine (GlcNAc) residues, two families of peripheral membrane proteins of the NPC have been distinguished: (a) O-linked glycoproteins which contain several copies of a more or less degenerate pentapeptide motif XFXFG and (b) non-O-linked proteins which do not contain any repetitive sequence motifs. In addition, p62 contains a COOH-terminal α -helical coiled-coil domain, and NUP153 harbors four 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 protein family are unique. In the case of Tpr/p265, it contains a ~1600-residue-long α -helical coiled-coil domain near its NH₂-terminal end. For more information about these proteins, see Table I and references therein. FIG. 3. Schematic diagram of the domain architecture of the cloned and sequenced yeast NPC proteins deduced from their amino acid sequences. Depending on the occurrence of highly repetitive motifs in their amino acid sequences, three families of yeast NPC proteins have been distinguished: (a) the XFXFG family which contains several copies of a more or less degenerate pentapeptide motif XFXFG clustered in the central part of each protein; (b) the GLFG family whose members contain several copies of a degenerate tetrapeptide motif GLFG within their NH₂-terminal domain; and (c) the third family includes the yeast nucleoporin interacting component NIC96 which does not contain any repetitive sequence motifs and forms a complex with NSP1 and NUP49 (Grandi *et al.*, 1993). Three members of the GLFG family—NUP100, NUP116, and NUP145—contain related domains including an RNA-binding motif. For more information about these proteins, see Table I and references therein. FIG. 5. Schematic diagram summarizing the immunolocalization of characterized NPC protein epitopes within the 3-D architecture of the consensus model of the NPC. The major structural components of the NPC include the basic framework (i.e., the spoke complex), the central plug or channel complex, the cytoplasmic and nuclear rings, and the cytoplasmic filaments and nuclear basket. The 52-MDa basic framework of the NPC has been adapted from a conical tilt reconstruction of negatively stained detergent-released NPCs (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). 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 spoke complex (see Hinshaw *et al.*, 1992). The central plug or channel complex has been modeled as a transparent ellipsoidal particle to indicate the fact that its definite structure remains elusive. Tpr/p265, CAN/NUP214/p250, and NUP180 exhibit epitopes residing in the cytoplasmic filaments (see Figs. 4c, 4d, and 4e; Byrd *et al.*, 1994; Panté *et al.*, 1994; Wilken *et al.*, 1993), whereas NUP153 exhibits an epitope at the terminal ring of the nuclear basket (see Fig. 4b; Panté *et al.*, 1994). p62 epitopes are exposed at both the cytoplasmic and nuclear periphery of the central plug or channel complex (see Fig. 4a). The transmembrane glycoprotein gp210 exhibits several epitopes in the lumen of the NE (Greber *et al.*, 1990) where based on its topology most of its mass resides (see Fig. 1; Greber *et al.*, 1990). Epitopes for the transmembrane glycoproteins POM121 and POM152 are shown too, but these should not be taken literally as their exact localization remains to be determined. In contrast to gp210, most of the mass of POM121 is predicted to reside within the NPC proper (Hallberg *et al.*, 1993). Very much like gp210, most of the mass of yeast POM152 is predicted to reside in the lumen of the NE (Wozniak *et al.*, 1994).

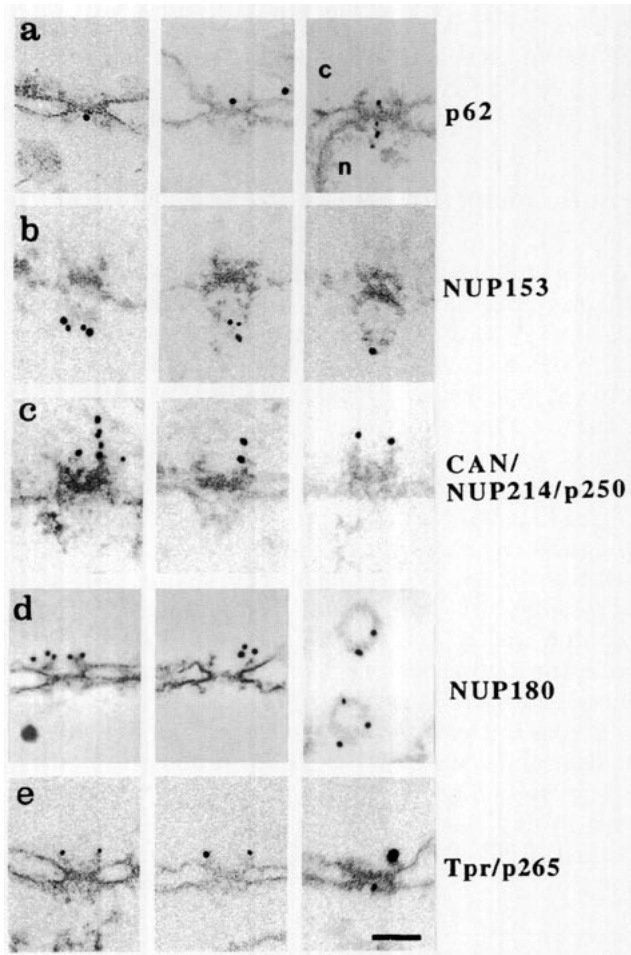


FIG. 4. Immunolocalization of NPC proteins. Selected examples of labeled NPC cross-sections revealing localization of epitopes of (a) p62 at both the cytoplasmic and nuclear periphery of the central plug or channel complex (using the monoclonal RL31 antibody; Guan *et al.*, manuscript in preparation); (b) NUP153 at the terminal ring of the nuclear basket (using an anti-peptide antibody; Panté *et al.*, 1994); (c) CAN/NUP214/p250 at the cytoplasmic filaments (using a polyclonal anti-p250 antibody; Panté *et al.*, 1994); (d) NUP180 at the cytoplasmic ring or cytoplasmic filaments (using affinity-purified antibodies from a serum of a patient with overlap connective tissue disease; Wilken *et al.*, 1993); and (e) Tpr/p265 at the cytoplasmic filaments (using the monoclonal RL30 antibody; Byrd *et al.*, 1994). In all cases, the antibodies were directly conjugated to 8-nm colloidal gold, and the NEs were labeled prior to embedding and thin sectioning. The rightmost example of (e) is an NPC that has been double-labeled with RL31 (anti-p62) conjugated to 8-nm colloidal gold and RL30 (anti-Tpr/p265) conjugated to 14-nm colloidal gold. (a and e) Rat liver NEs; (b, c, and d) *Xenopus* oocyte NEs; c marks the cytoplasmic, and n marks the nuclear side of the NE. Scale bar, 100 nm.

sion 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 particular NPC component(s). Recently, more specific localiza-

tion of NUP153 has been achieved. Using an antibody raised against an extract of nuclear matrix proteins that by immunoblotting recognized NUP153, Cordes *et al.*, (1993) have localized this protein to intranuclear NPC-attached filaments which, among other structures, may represent nuclear baskets that have been disrupted during sample preparation. More specifically, Panté *et al.*, (1994) have identified NUP153 as a constituent of the nuclear basket with at least one of its epitopes residing in the terminal ring (see Fig. 4b). This localization for NUP153 together with its four zinc finger motifs is consistent with the stabilizing effect of Zn^{2+} on the nuclear baskets (Jarnik and Aebi, 1991): in the presence of 0.5 mM $ZnCl_2$ well-formed baskets are observed, whereas when divalent cations are chelated by 2 mM EGTA or EDTA, the nuclear baskets become destabilized and are disrupted. Surprisingly, if after destabilization by EDTA or EGTA divalent cations are again added, 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 have a role in maintaining the structural integrity of the nuclear baskets and therefore could be directly involved in the active transport of proteins, RNAs, or RNP particles through the NPC. We are currently testing this hypothesis using an anti-peptide antibody against the zinc finger motifs.

CAN/NUP214 has also been expressed as a fusion protein, and a polyclonal antibody raised against this fusion protein labeled the cytoplasmic periphery of the NPC (Kraemer *et al.*, 1994). However, this labeling was not specific enough to identify CAN/NUP214 with a distinct NPC component(s). Using a monoclonal antibody called QE5, Panté *et al.*, (1994) have identified a ~250-kDa O-linked NPC glycoprotein, termed p250, in extracts of BHK cells. As illustrated in Fig. 4c, a polyclonal antibody raised against p250 specifically labeled the cytoplasmic filaments of *Xenopus* oocyte NPCs. On immunoblots, p250 is also recognized by the monoclonal RL1 antibody used by Snow *et al.*, (1987) (B. Burke and R. Bastos, personal communication). In addition, p250 is recognized by polyclonal antibodies raised against NH₂- and COOH-terminal peptides synthesized based on the deduced amino acid sequence of cloned human CAN (B. Burke and R. Bastos, personal communication). Taken together, these data show that p250 corresponds to the originally identified ~210-kDa O-linked NPC glycoprotein (Snow *et al.*, 1987) that has recently been demonstrated to represent a homologue of human CAN and termed CAN/NUP214 (Kraemer *et al.*, 1994).

Epitopes of at least two other—both non-O-linked—NPC proteins have been localized to the cy-

toplasmic filaments of the NPC: (i) NUP180 (Fig. 4d; Wilken *et al.*, 1993), and (ii) Tpr/p265 (Fig. 4e; Byrd *et al.*, 1994). Based on the ~1600-residue-long α -helical coiled-coil domain of Tpr/p265 (see Fig. 2b; Mitchell and Cooper, 1992), it is conceivable that this protein, together with CAN/NUP214/p250 (see Fig. 4c; Panté *et al.*, 1994) and NUP180 (Wilken *et al.*, 1993), forms the backbone of the cytoplasmic filaments.

As summarized in Fig. 5, epitopes of six NPC proteins have thus far been localized within the 3-D NPC architecture. However, we want to emphasize that due to the unknown native conformation of these proteins, it is difficult to map their entire extent within the NPC. For example, whereas at least one epitope of NUP153 resides in the terminal ring of the nuclear baskets (see Fig. 4b), this protein could be part of the terminal ring and/or the filaments forming the nuclear baskets. Similarly, due to their size, the constituent proteins of the cytoplasmic filaments, i.e., CAN/NUP214/p250, Tpr/p265, and NUP180, beyond spanning the length of a cytoplasmic filament, might extend into the cytoplasmic ring.

As can be appreciated from Fig. 5, with the exception of the transmembrane glycoprotein gp210, the epitopes of NPC proteins that have thus far been identified with distinct structural components of the NPC are localized at either the cytoplasmic or the nuclear periphery of the NPC. Therefore, the constituent proteins of the 52-MDa basic framework of the NPC (i.e., the spoke complex) remain to be identified. Thus far, gp210 is the only NPC protein which has been identified as a constituent of the basic framework of the NPC (Greber *et al.*, 1990).

ISOLATION AND CHARACTERIZATION OF DISTINCT SUBCOMPLEXES OF THE NPC

When assembled in the NPC, several NPC proteins may mutually interact to form distinct subcomplexes. In vertebrate species, it was first reported that some of the soluble NPC proteins contained in *in vitro* nuclear reconstitution extracts from *Xenopus* oocytes form a supramolecular complex with a molecular mass of 254 kDa which contains p68, the *Xenopus* homologue of rat p62, together with several other NPC proteins (Dabauvalle *et al.*, 1990). The rat homologue of this supramolecular complex has also been isolated and characterized at the molecular level (Finlay *et al.*, 1991; Kita *et al.*, 1993; Buss and Stewart, 1995). It consists of p62 interacting with two other proteins of molecular mass 58 (p58) and 54 (p54) kDa. The estimated molecular mass of the p62 complex is 200–600 kDa with no consensus on its subunit stoichiometry (Finlay *et al.*, 1991; Kita *et al.*, 1993; Buss and Stewart, 1995). To resolve these ambiguities, we have devel-

oped a modified procedure to isolate the p62 complex from rat liver NEs (Guan *et al.*, manuscript in preparation). In addition to p58 and p54, p62 in this supramolecular complex is associated with a 45-kDa NPC protein having a peptide map similar to p58. However, p45 is only revealed when extreme caution is taken to avoid proteolysis during isolation. When examined in the EM after negative staining or glycerol spraying/rotary metal shadowing, this complex appears as a ~15-nm-diameter particle (Guan *et al.*, manuscript in preparation). We are currently investigating its molecular mass and subunit stoichiometry by quantitative scanning transmission EM (STEM).

Using the monoclonal QE5 antibody, Panté *et al.*, (1994) have identified two distinct NPC subcomplexes in extracts of BHK cells in addition to the p62 complex. While this antibody recognizes p62, NUP153, and p250 on Western blots, it immunoprecipitates three additional polypeptides, p54, p58, and p75, which were found to be associated with p62 (p54 and p58) and p250 (p75). Furthermore, in these extracts NUP153 existed as a homooligomer of ≥ 1 MDa, most likely representing an octamer. As several of its epitopes have been located to the terminal ring of the nuclear baskets (see Fig. 4b; Panté *et al.*, (1994), it is conceivable that the octameric NUP153 complex defines the basic framework of the eight-fold symmetrical terminal ring.

In yeast, Hurt (1990) has expressed the α -helical COOH-terminal domain of NSP1 and found that the fusion protein was targeted to yeast NEs. Based on this observation, he proposed that NSP1 might specifically interact with other yeast NPC proteins (Hurt, 1990). Indeed, NSP1 has now been shown to form a complex with three other proteins, NIC96, NSP49, and a novel yeast NPC protein of 54 kDa that has not yet been cloned and sequenced (Grandi *et al.*, 1993). Recently, a second subcomplex has been identified in yeast NPCs (Belanger *et al.*, 1994). It consists of NUP1 and NUP2 interacting with Srp1, the product of a gene previously identified as a suppressor of mutants defective in RNA polymerase I (Yano *et al.*, 1992).

CONCLUSIONS AND FUTURE PROSPECTS

By now 18—nine vertebrate and nine yeast—of the approximately 100 polypeptides of the NPC have been identified, characterized, and cloned and sequenced (see Table I). The epitopes of several of these NPC proteins have been localized to distinct structural components of the NPC by immuno-EM (see Fig. 4); thus, the molecular architecture of the NPC is slowly but definitely taking shape (Fig. 5). However, even if several copies (i.e., 8 or 16 because of the 822 symmetry of the basic framework of the NPC) of the proteins thus far identified reside in the

NPC, they represent only ~15% of the entire NPC mass. Therefore, we have to go a long way before the complete architecture of the NPC will be unveiled at the molecular level. Toward this goal, the recent success to bulk-isolate NPCs from yeast (Rout and Blobel, 1993) has opened the possibility of more systematically identifying the proteins constituent of yeast NPCs. Most importantly, this system offers the possibility of combining molecular genetics approaches with biochemical, structural, and functional analyses of the NPC.

The next step toward a more complete molecular architecture of the NPC has to delineate individual NPC proteins within the 3-D structure of the NPC and analyze their conformation and specific interactions with other proteins residing within distinct NPC components or subcomplexes. Moreover, to ultimately reconstitute functional NPCs *in vitro*, we also have to mass-isolate and molecularly characterize distinct NPC components or subcomplexes, e.g., the spoke complex, the cytoplasmic and nuclear rings, the cytoplasmic filaments, the nuclear basket, and the central plug or channel complex, determine their 3-D molecular architecture, and decipher their functional task(s) in passive or mediated nucleocytoplasmic transport. Toward this goal, several NPC subcomplexes have now been identified: i.e., the p62 complex, the p250-p75 complex, the NUP153 homooligomer, the yeast NSP1 complex, and the yeast NUP1-NUP2-Srp1 complex. However, the structural and functional analyses of these NPC subcomplexes at the molecular level has only begun and will require joint efforts to be completed within a reasonable time frame.

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