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# ARCHITECTURE OF THE NUCLEAR PORE COMPLEX AND ITS INVOLVEMENT IN NUCLEOCYTOPLASMIC TRANSPORT

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**Abstract**—Recent structural analyses of the nuclear pore complex (NPC) have described in some detail the numerous sub-domains which make up this supramolecular assembly. Three dimensional image analysis of detergent-extracted NPCs reveals that the NPC framework is made up of spoke units, each containing four major domains, arranged with 822 symmetry. As shown by freeze-drying/metal shadowing techniques, attached to this framework are several peripheral components including particles and fibers on the cytoplasmic face and a cage-like structure on the nucleoplasmic face. While it is known that traffic between cytoplasm and nucleus occurs exclusively through the nuclear pore, the specifics of NPC involvement in such traffic remain unknown at present. Proteins destined for the nucleus contain nuclear localization sequences (NLSs). Several proteins have been identified which bind to these NLSs and may act to direct these proteins to the NPC, either releasing them prior to or remaining attached during translocation through the NPC. These NLS-binding proteins have been localized to the cytoplasm, nucleus, nuclear envelope and nucleolus, suggesting some of these proteins transverse the NPC bound or unbound to NLS-containing proteins.

The exchange of material between the cytoplasm and nucleus of eukaryotic cells occurs exclusively through a large (125 MDa) macromolecular assembly which spans the double bilayer of the nuclear envelope. This assembly, known as the NPC† allows small molecules (less than 90 Å) to passively diffuse between the nucleus and cytoplasm while large macromolecules (up to 250 Å) are actively and selectively transported bidirectionally (for reviews see: Refs. 1, 2). Cytoplasmic proteins which are to be directed to the nucleus contain NLSs which consist of predominantly small stretches of basic amino acids (for review see Ref. 3). Several laboratories have identified proteins that bind to these NLSs and may direct the NLS-containing proteins to the NPC (see references in Table 1).

The biochemical composition of the NPC is still poorly understood due primarily to the lack of an adequate isolation procedure. It is estimated that only 10% of the NPC proteins have been identified [4]. The majority of the known NCP proteins are members of a family of glycoproteins that contain O-linked *N*-acetylglucosamine residues [5–9]. These glycoproteins have been localized as peripheral components of the NPC on both the cytoplasmic and nucleoplasmic sides of the nuclear envelope [5, 6, 8, 9], and have been shown to be required for transport of material through the NPC [10]. In addition to the above glycoproteins, a single transmembrane glycoprotein gp210, has been identified, sequenced and localized [11, 12]. The majority of the mass of gp210 is found within the lumen of the nuclear envelope near the NPC where it has been proposed to act as an anchor for the NPC in the nuclear envelope [11, 12].

### *Structure of the NPC*

During the past few years there has been an acceleration of work on the structure of the NPC. Early studies involving thin-sections of the nuclear envelope revealed that the NPC has 8-fold rotational symmetry and that the inner and outer nuclear envelope is continuous around the NPC [13, 14]. In more recent years, the sub-structure of the NPC has

Table 1. Nuclear localization sequence binding proteins

| Species | Molecular weight (kDa) | Location* | Reference |
|---|---|---|---|
| Rat | 70,60 | N&C, N&C | 28 |
| | 140,100,70,55 | N,C,C,N | 30 |
| | 56,57,65,74 | N,N,N,N | 33 |
| | 60,67,60,53,47 | N,N,N,N,N | 32 |
| | 140,55 | NO,N | 37 |
| | 69 | N | 40 |
| | | | 41 |
| Human | 38 | NO | 42 |
| | 66 | N | 31 |
| Yeast | 140,95,70,59 | N,N,N,N | 34 |
| | | | 35 |
| | 67 | NO | 38 |
| | | | 39 |

* Location: N, on nuclei; C, in cytoplasm; NO, in nucleoli.
Similar proteins found in rat, yeast and human.

* Tel. 619 554 9889; FAX 619 554 6112.
† Abbreviations: NPC, nuclear pore complex; NLS, nuclear localization sequence.

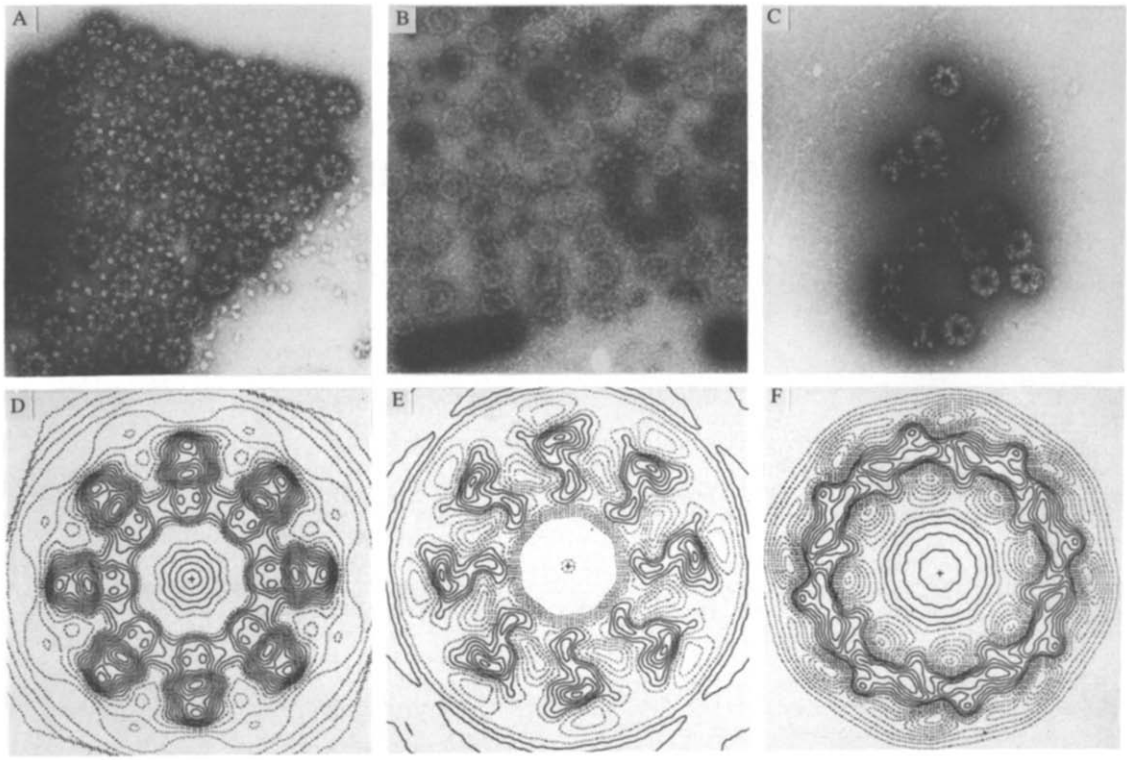


Fig. 1. Electron images (a–c) and 2-dimensional electron density maps (d–f) of detergent-released components of the nuclear envelope. (a) A patch of face on views of the intact NPC; (b) rings and intermediate structures; and (c) face on, edge and oblique views of the intact NPC. All specimens are seen embedded in negative stain. Averaged 2D projection maps of the NPC (d) intermediate structure (e) and ring (f).

been revealed by 2-dimensional projection maps calculated from electron images of negatively stained and frozen hydrated samples [15–19]. In addition, two separate groups have calculated 3-dimensional maps of the NPC, again from electron images [16, 20]. Finally, the surface topology, including numerous peripheral appendages, has been revealed by metal shadowing/freeze-drying [21–23] and scanning electron microscopy [24–26].

Examination by electron microscopy of negatively-stained, detergent-treated nuclear envelopes isolated from xenopus oocytes reveals four distinct structures closely associated with the nuclear membrane: (1) face on views of NPCs; (2) edge-on views of the NPC; (3) lightly staining rings; (4) and structures that stain with about one-half the intensity of the NPC which have been termed "intermediate" structures (see Fig. 1a, b and c) [16]. 2D projection maps of the NPC determined from the face on views reveal eight spokes, giving the NPC its 8-fold rotational symmetry (Fig. 1d). Each spoke is subdivided into three stain-excluding regions, with the outer-most region located within the lumen of the nuclear envelope. Based on the location of this outer region, this lumenal domain of the NPC may correspond to the transmembrane glycoprotein gp210. The inner regions are continuous with each other, encircling a ~420 Å diameter hole while the outer regions are connected by weak radial arms. The 2D map determined from the intermediate structures is similar in size to the NPC; however, each of the eight spokes display an 'S'-shaped motif (Fig. 1e). The 2D map of the rings consists of eight subunits, with each subunit appearing to have a pseudo 2-fold axis (Fig. 1f). This suggests that the ring may actually consist of 16 identical units.

The 3D map of the NPC calculated from negatively-stained, detergent-extracted NPCs by the random conical tilt method [27] suggests that, in addition to the large central channel, there exists eight peripheral channels of apporoximately 100 Å between the spokes that may function as backup channels for passive diffusion (Fig. 2) [16]. Analysis of the 3D map as well as face on and edge-on views of the NPC suggest the basic framework of the NPC is 2-fold symmetric about the plane of the membrane. Sections from the top half of the 3D map are very similar to sections from the bottom half but with the opposite hand, giving the NPC 822 symmetry. Comparisons of the 2D maps of the rings and intermediate structures with sections of the 3D map indicate that the rings originate from the outer faces of the NPC, and the intermediate structure may represent one half of a NPC. Sections of the 3D map on either side of the central plane display the 'S' motif seen in the projection maps of the intermediate structure, and slices at the periphery of the 3D map closely resemble the 2D map of the ring structures. Based on density peaks in the 3D map, the architecture of the NPC may be divided

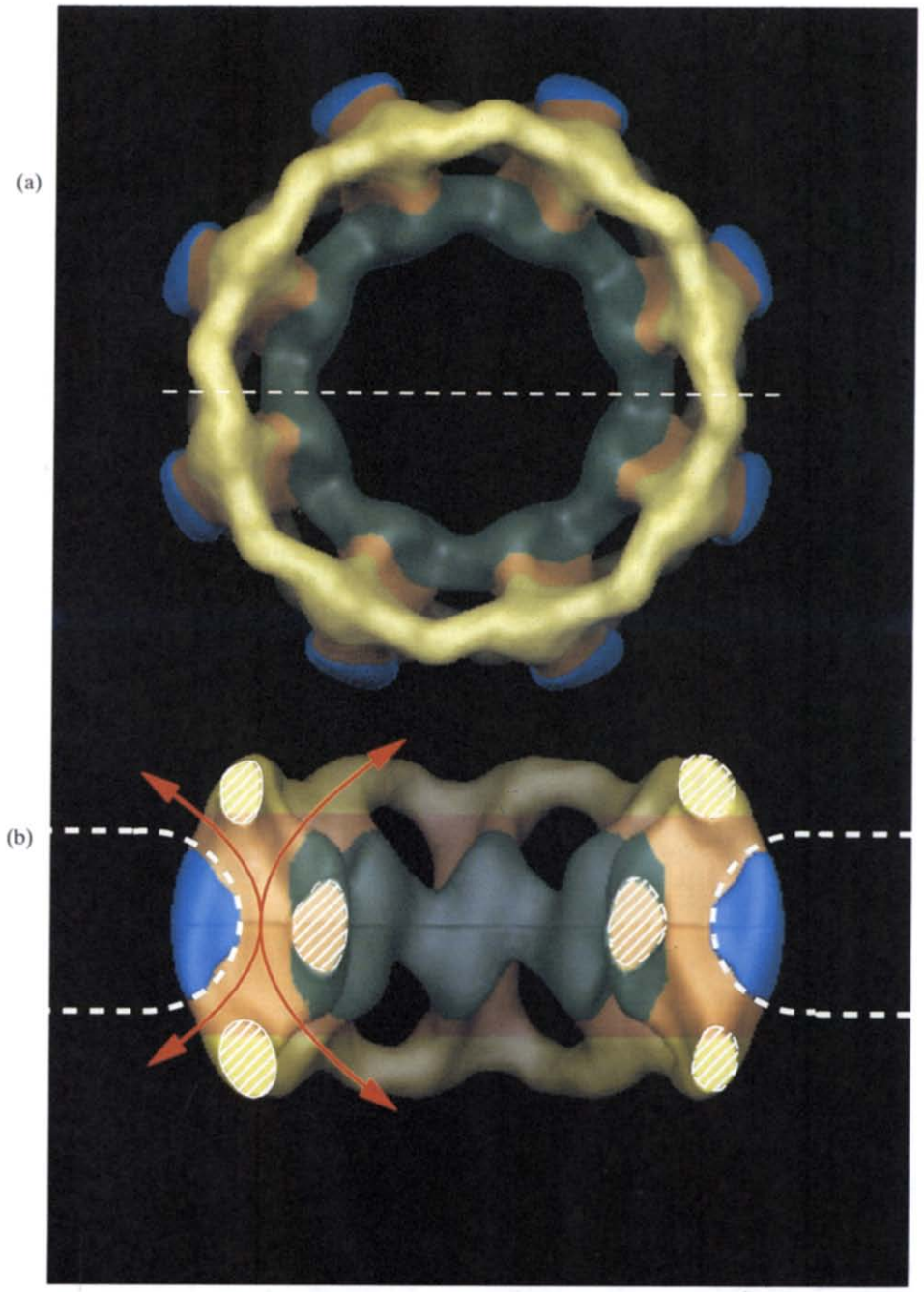


Fig. 2. Surface views of 3D electron density map of NPC calculated from negative stained samples and displayed by SYNU [45]. (a) Top and (b) cut away side view of the NPC. The dotted line in (b) represents the position of the nuclear envelope, while the double-headed arrows show the possible alternate routes for passive diffusion through the peripheral channels. The hatched areas indicate the cut ends of the rings and annular regions of the NPC. The surfaces contoured to include approximately 100% of the expected mass of the NPC. The four subunits of the NPC are color-coded as follows: annular subunits are green, rings are yellow, lumenal subunits are blue and the rest of the NPC, including the column subunits, is tan.

into four subunits: ring, column, lumenal and annular (Fig. 3). The ring subunits appear to be easily removed from the NPC as demonstrated by treatment with a mild detergent. The annular subunits connect adjacent spokes by diagonal links that not only hold the spokes together but also join the top half to the bottom half of the NPC. If the intermediate structures are indeed half pores then this connection is crucial for maintaining the structural integrity of the NPC. The lumenal subunits, as mentioned above, are located in the lumen of the nuclear membrane and help anchor this large macromolecule to the

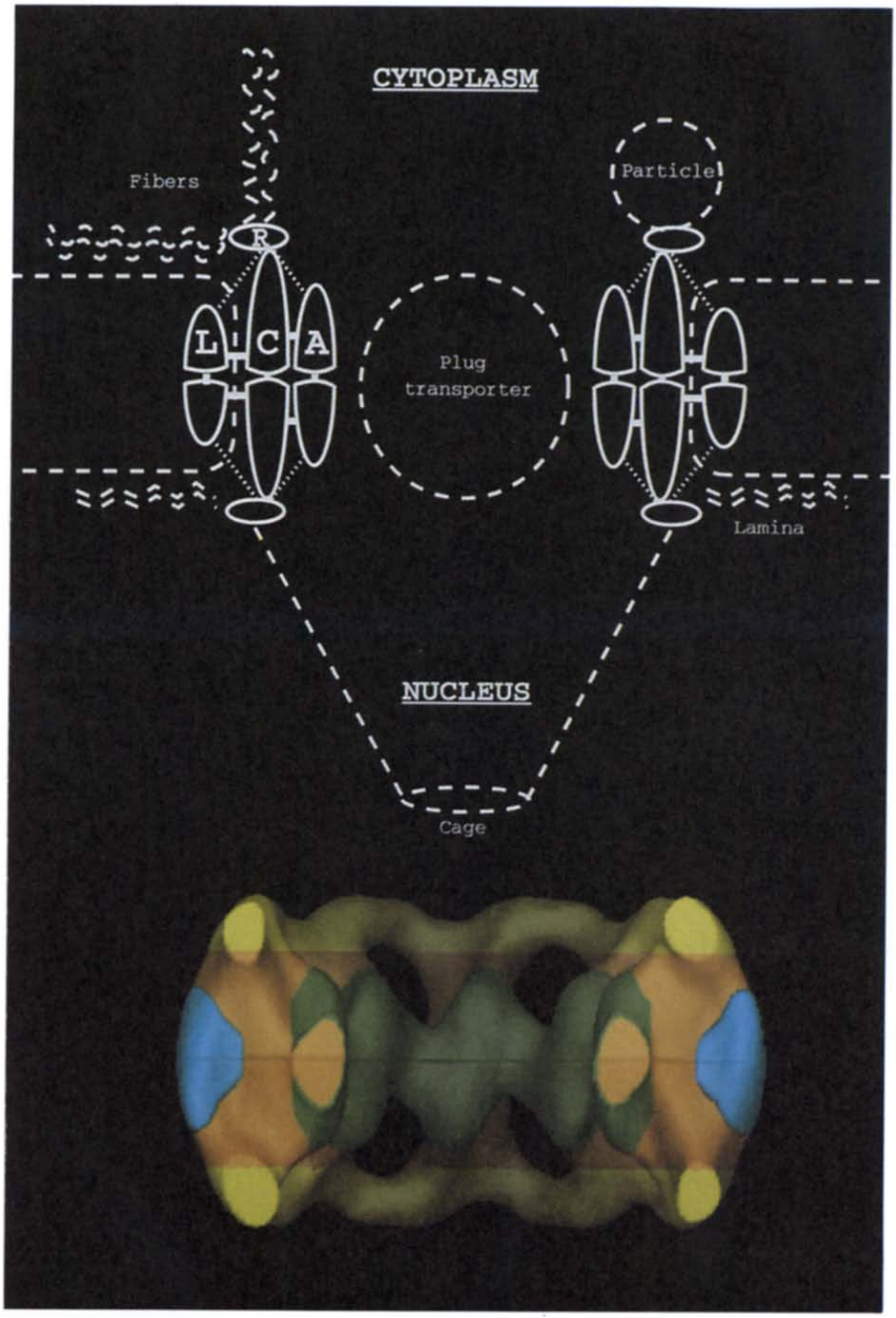


Fig. 3. Schematic diagram of the NPC including the basic framework (solid lines) and all its peripheral components. The four architectural subunits of the NPC are identified as R (ring), L (lumenal), C (column) and A (annular) on both the schematic diagram and the cut-away side view of the molecular surface of the 3D map of the NPC.

membrane. Finally the column subunits coordinate the binding of all the subunits.

Recently, Akey and Radermacher [20] have calculated 3D maps of the NPC, both in the membrane and with the membrane removed by detergent extraction, from frozen hydrated samples. Overall these maps are very similar to the 3D map calculated from negative stain preparations. In addition to the basic framework of the NPC described above, this recent 3D map from detergent-extracted specimens also includes a central plug (or transporter) and cytoplasmic particles residing between the spokes. The transporter, which Akey and Radermacher suggest is a macromolecular transport channel, has a tripartite structure and appears hollow in the center. Just as with the negatively stained 3D map described above, sections through the top and bottom halves of this 3D map are similar but have

the opposite hand. Excluding the cytoplasmic particles, this again suggests that the NPC is 2-fold symmetric about the plane of the membrane. This structure also clearly shows the radial arms that connect the lumenal subunits of the NPC. Comparisons between the membrane-associated and the detergent-extracted 3D structures reveal slight differences that suggest the spokes may be flexible enough to allow for conformational changes. Akey and Radermacher also suggests that between the transporter and spokes are internal channels that allow for passive diffusion. These channels differ from the peripheral channels described above in that they are positioned within the large central channel rather than running between each spoke.

To examine the surface topography of the NPC, freeze-drying/metal shadowing techniques and scanning electron microscopy have been used. Utilizing low-voltage scanning EM, Hans Ris first showed the existence of a cage, or basket-like, structure on the nucleoplasmic side of the NPC [25, 26]. Eight long filaments extend out from each of the NPC spokes into the nucleoplasm by as much as 100 nm and end in a distal ring. In addition, Ris also revealed shorter fibers on the cytoplasmic side of the NPC. Freeze-drying methods have also shown the cages on the nucleoplasmic side and fibers or fibrils projecting from the cytoplasmic face or forming links between adjacent NPCs [21, 23]. Goldberg and Allen [24] further demonstrated the existence of the cages by high-resolution scanning EM (HRSEM) and also showed that occasionally a lattice structure is attached to the distal ring of the cage. It is unclear if this lattice covers the entire nuclear envelope or is only found in regions of high membrane curvature. The lattice may act as a sieve to retain large macromolecules or a physical bridge to maintain the integrity of the nuclear envelope.

*NLS-binding proteins*

Proteins destined for the nucleus either contain NLSs or bind to other proteins that contain such sequences. The identification of NLSs suggested that there exists receptors that bind to these specific sequences. Cross-linking studies using the SV40 large T antigen signal sequence [28–33]; blotting with anti-idiotype antibodies against SV40 signal sequence [34–36]; blotting with the SV40 signal sequence itself [37]; ligand blotting [38, 39]; and affinity chromatography [40–42] revealed several cytoplasmic and nuclear proteins that bind to NLSs (Table 1). What role, if any, these proteins play in nucleocytoplasmic transport is unknown. Some of these proteins may bind to several small peptides and have a more general function than nucleocytoplasmic transport (such as the chaperonin family of proteins). For example, it has been shown that a lumenal ER protein with a molecular weight of 55 kDa, identified as protein disulfide isomerase (PDI), artificially *in vitro* binds to NLSs [43]. However, NLS-binding proteins which have a specific function in nucleocytoplasmic transport have also been identified. Adam and Gerace [29] have shown that on the addition of two proteins of 54 and 56 kDa isolated from bovine erythrocytes, nuclear transport is enhanced 2–3-fold. It has also been shown that transport of NLS-containing proteins which are sufficiently small that they could potentially diffuse into the nucleus is arrested by chilling or energy depletion. This suggests that the presence of a NLS prevents diffusion of such a protein into the nucleus, and that this inhibition of diffusion may also involve cytoplasmic receptors [44].

*Future prospects*

The structure of the NPC including its peripheral components is a highly complex assembly which potentially contains hundreds of proteins. In the past, the identification of these proteins has been slow, but recently, yeast NPCs have been purified and information concerning their protein composition should soon be forthcoming. Once more NPC proteins have been identified, their location within the NPC can be discerned by immunolocalization and mutant studies in combination with structural studies described above. For example, it should be possible using immunolabeling techniques to identify individual proteins as being components of a specific architectural subunit (ring, column, lumenal, annular) of the intact NPC. Additionally, it may be possible to identify proteins important to the integrity of the NPC by immunolabeling the three distinct structures (ring, intermediate and intact NPC) seen in electron micrographs. Finally, isolation and biochemical analysis, as well as functional mutants of NPC proteins whose location within the NPC are known, will assist in providing clues to their role(s) in nucleocytoplasmic transport.

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