

Structural and functional organization of the nuclear envelope

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The double-membrane nuclear envelope is punctuated by pores where the two membranes are joined. These pores are stabilized by the elaborate nuclear pore complex, which is anchored to the inner membrane by the nuclear lamina, as well as to other nuclear and cytoskeletal structures. Recent experiments have identified proteins involved in the stability of this organization as well as in the function of the nuclear pore complex, which we relate here to newer aspects of nuclear envelope structure.

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Introduction

The nuclear envelope (NE) is not just a boundary to the nucleus, but is an elaborate organelle that forms the interface between the nuclear and cytoplasmic compartments. It is involved not only in nucleo-cytoplasmic communication, via the nuclear pore complexes (NPCs), but also in organizing nuclear contents, in maintaining nuclear shape, in DNA replication and nuclear growth, and also in protein synthesis and processing. It may also have a role in RNA processing and probably in other processes that have yet to be imagined. The NE interacts with cytoplasmic and nuclear structures and is highly dynamic (for reviews, see [1–6] and references therein). Here, we have brought together much of what is known about the fine structure of the NE and relate this to new functional and structural studies. Some of what we know about NE structure from electron microscopy (EM) is summarized in Figure 1. The NE consists of several components that are discussed individually below.

The membranes of the nuclear envelope

The outer nuclear membrane (ONM) is a cytoplasmically opposed lipid bilayer that is connected to and resembles the rough endoplasmic reticulum, with functional ribosomes attached. The inner nuclear membrane (INM), although connected to the cytoplasmic membrane, does not resemble the rough endoplasmic reticulum, and contains its own set of proteins. It is lined with a network of lamin filaments (Fig. 2) that is sometimes seen as a square array with a 52 nm repeat [7] or a ~20 nm repeat ([8•]; MW Goldberg, unpublished data).

The filaments lie close to the membrane and, although they are not integral membrane proteins, B-type lamins have a lipid modification that inserts into the membrane [9]. There are also integral membrane proteins such as lamina-associated polypeptides [10] and p58 [11], which may interact with, or anchor, lamins, chromatin or other structures to the INM. NPCs are also anchored in the lamina (Fig. 2) but what mediates this interaction is not known (but see below). The INM is a dynamic membrane [3], that is able to grow during S phase and vesicularize at mitosis, and that seems to bud during viral infection [12].

Thin-section EM has shown that the INM and ONM are connected at the pores. The membrane present in the pore is called the pore membrane; it is associated with integral and peripheral membrane proteins of the NPC and may provide a route for transferring integral membrane proteins from the INM to the ONM or vice versa [3].

The nuclear envelope lumen

The NE lumen is the aqueous domain enclosed by the NE membrane. It overlaps functionally with the lumen of the rough endoplasmic reticulum, but may carry out unique functions. It provides the environment for one side of the ONM, INM and pore membrane so that integral proteins of these membranes have luminal domains [10,11]. These proteins may simply anchor structures such as the NPC and lamina, or, more interestingly, some of them may provide a functional connection or communication between the lumen and the nucleus,

Abbreviations

3D—three-dimensional; EM—electron microscopy; FEISEM—field emission in-lens scanning EM; INM—inner nuclear membrane; NE—nuclear envelope; NEL—NE lattice; NPC—nuclear pore complex; ONM—outer nuclear membrane.

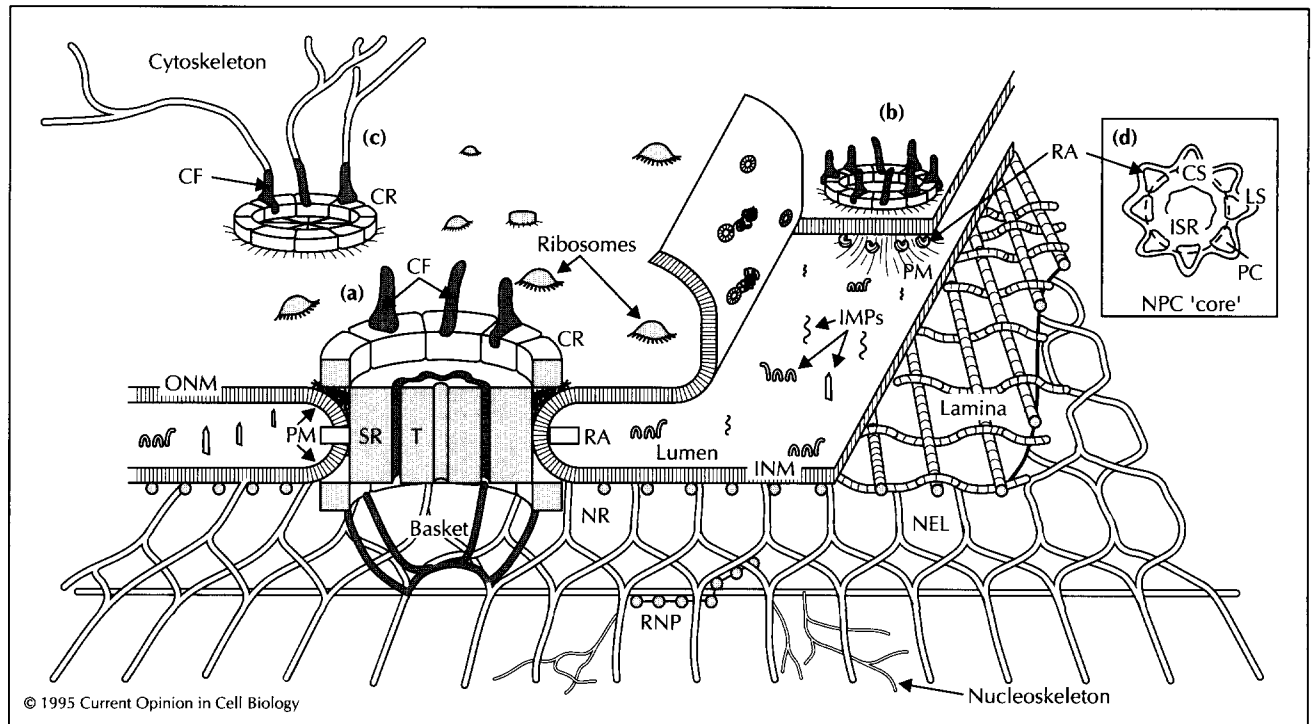


Fig. 1. Cut-away diagram of the nuclear envelope. One NPC is shown cut in half (a), some detail is omitted (see [17^{**},18]), and part of the ONM is 'peeled back' to give a proposed luminal view of another (b). A third is seen as from the cytoplasm (c). Cytoskeletal and nucleoskeletal attachments are hypothetical. CF, cytoplasmic filaments; CR, cytoplasmic ring; IMPs, integral membrane proteins; INM, inner nuclear membrane; NEL, nuclear envelope lattice; NR, nucleoplasmic ring; ONM, outer nuclear membrane; PM, pore membrane; RA, radial arms; RNP, ribonucleoproteins, attached to NEL; SR, spoke ring; T, transporter. The inset (d) depicts a cross-section through the NPC 'core': CS, central spoke domain; ISR, inner spoke ring; LS, luminal spoke domain; PC, peripheral channel. The dashed line indicates the possible position of the pore membrane.

the NPC, or the cytoplasm. Such a role for the NE lumen in NE function was recently hinted at when it was shown that mitotic NE vesicles possess inositol triphosphate receptors, transmembrane Ca^{2+} channels that are responsible for localized, transient increases in Ca^{2+} concentration by releasing membrane-contained Ca^{2+} stores in response to an inositol triphosphate signal [13^{**}]. Release of Ca^{2+} from these vesicles is necessary for vesicle fusion during NE assembly [13^{**}]. Although the function of NE inositol triphosphate receptors during interphase is not known, they could influence nuclear events or NPC transport. Many nuclear enzymes require Ca^{2+} , with the nearest Ca^{2+} store to their site of action being the NE. Although the NPCs appear to be freely permeable to small molecules [14], the NE does seem to be able to affect nuclear Ca^{2+} concentration [15], and patch clamp experiments have indicated the presence of ion channels in the NE, although it was suggested that NPCs could serve this role [16].

The nuclear pore complex

The NPC is attached to the pore membrane via several integral membrane proteins. It has a modular structure and each component will be discussed below.

The NPC 'core'

Core structure

Three-dimensional (3D) reconstructions from transmission EM images of ice-embedded [17^{**}] or negatively-stained NEs [18], and direct visualization of dismantled NEs by field emission in-lens scanning electron microscopy (FEISEM; MW Goldberg, unpublished data) have indicated the existence of a peripheral ring structure within the lumen in the central plane of the pore membrane. The ring consists of eight loops (termed radial arms; Fig. 1d) joined together by eight 'spokes' which traverse the pore membrane. 3D reconstructions [17^{**},18] have identified three domains of each spoke: the luminal spoke, found in the lumen, joins the radial arms; the 'central spoke' domain may be the transmembrane segment; and the spokes are then joined together at the inner spoke domain by the 'spoke ring'. These structures together form the core of the NPC (Fig. 1).

Core proteins

Three transmembrane proteins have been identified which are seen in EM thin sections to localize to the NPC core. The glycoprotein gp210 has 95% of its mass in the lumen, a single transmembrane domain and a short carboxyl terminus which may be part of the NPC core within the pore itself [19]. Antibody

labelling [19] shows that gp210 is at a distance from the centre of the NPC, deep in the lumen, and might therefore be a component of the radial arms. It is N-glycosylated by high-mannose sugar groups on the luminal domain, a characteristic of the luminal or extracellular domains of many membrane proteins, for uncertain reasons [19]. The largely luminal position suggests that gp210 has a function in the lumen. The radial arms, which may contain gp210, may simply be there to hold the spokes in place within the pore domain, like a rubber band. However, introduction of an antibody to gp210 into the NE lumen inhibits transport through the NPCs [20]. This may be because distortion of the radial arms by the antibody causes rearrangement of other proteins attached to it, resulting in distortion of components of the transport machinery located towards the centre of the NPC [20]. Alternatively, the antibody could inhibit some event in the lumen which is necessary for transport. gp210 could be part of either a transmembrane ion channel (it has been proposed that cations modulate NPC basket structure [21]; see below) or some other mechanism involved in lumen–NPC communication. Indeed, it has been shown recently that depletion of calcium from the lumen of the endoplasmic reticulum and NE inhibits both passive diffusion and active transport through the NPC, suggesting that luminal calcium somehow affects NPC structure or activity [22].

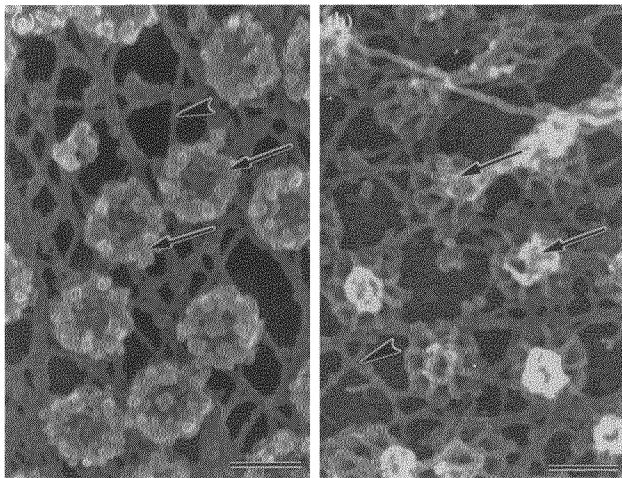


Fig. 2. Cytoplasmic (a) and nucleoplasmic (b) views of detergent-extracted *Xenopus* oocyte NEs imaged by FEISEM showing lamin fibres (arrowheads) attached to NPCs (arrows). Bar 100 nm.

The yeast protein Pom152 also has a single transmembrane domain and is thought to be situated mostly in the lumen [23^{*}]. Immunogold localization positions it close to the pore membrane, suggesting that Pom152 may be part of the spoke assembly, although, as the epitope against which the antibody was directed is close to the transmembrane domain, the position of the bulk of the protein is not known. Its function is also unknown: deletion mutants are viable and have no detectable structural defects [23^{*}].

Rat Pom121 localizes to the same region, so may be part of the spokes, but is thought to be mostly on the pore side of the membrane, rather than in the lumen [24^{*}]. Although it is substantially different from Pom152 and probably located mostly on the opposite side of the membrane, Pom121 shares a 19 amino acid long stretch of sequence homology with Pom152, suggesting that the two proteins may have overlapping properties or functions.

Peripheral channels

Between each radial arm and the spoke ring there are gaps that have been referred to as 'peripheral channels' [18] (Fig. 1d). It has been suggested that these channels are the route for diffusion of molecules to and from the nucleus, as their dimensions are consistent with the upper size limit for diffusion into the nucleus from the cytoplasm (see [14] for review). However, 3D reconstructions of membrane-associated NPCs (rather than of isolated NPCs) suggest that the membrane is at a position close to the inner spoke ring that would place the channels within the NE lumen and therefore make them inaccessible for nucleo–cytoplasmic diffusion [17^{**}]. This is supported by FEISEM images of proteolysed NEs where the cytoplasmic ring has been removed to show that the membrane is closely associated with the spoke ring [8^{**}] and that no peripheral channels are visible unless the membrane is removed by detergent (MW Goldberg, unpublished data). It is possible, however, that integral membrane proteins move from the INM to the ONM, and vice versa, along the pore membrane that is routed through these channels [3], allowing control of this movement by the NPC.

Cytoplasmic ring

The cytoplasmic ring is attached to the spoke assembly by vertical supports (not shown in Fig. 1) [17^{**},18]. The cytoplasmic ring consists of eight equal bipartite subunits (seen in Fig. 3 and Fig. 4). It has a diameter of ~120 nm (in amphibian oocytes; [25]), a height of ~15 nm [8^{**}], a mass of ~32 MDa [26] and stands above the level of the ONM [8^{**}]. Its protein composition and function are unknown. Much of the underside of the ring may be in contact with the ONM and so could be involved in organizing this membrane as it enters the NPC domain. As discussed below, it could function to hold down the ONM (like a ring of eight rivets) and ensure that it connects to the pore membrane without flowing over the NPC. A possible component of the cytoplasmic ring is a peripheral membrane protein called Nup180, recognized by an autoimmune antibody from a patient with overlap connective tissue disease [27^{**}]. Immunogold labelling of transverse and oblique sections is consistent with its labelling the cytoplasmic ring. Microinjecting anti-Nup180 into *Xenopus* oocytes does not affect NPC transport [27^{**}], supporting a role for the ring in maintaining NPC structure, rather than in

the actual transport mechanism. On the other hand, it has also been suggested [28] that p62, which is essential for transport, is also part of this ring. p62 is a nucleoporin, that can form filaments *in vitro* and contains a coiled-coil carboxy-terminal rod domain which strongly interacts with p54, forming an octamer complex ($2 \times p62$, $4 \times p54$ and $2 \times p58$). Epitopes in the rod domain are masked *in situ* whereas the amino-terminal domain is exposed, suggesting that the amino-terminal head protrudes into the central channel [29]. It was suggested [28] that the ability of p62 to form filaments could be used to form part of the cytoplasmic ring when in a complex with the other proteins in the context of the NPC.

Nucleoplasmic ring

The nucleoplasmic ring (Fig. 4b) is similar in structure to the cytoplasmic ring but may be less massive (~21 MDa) [26] and more sensitive to proteolysis [8**]. Its composition and function are not known but recent genetic experiments in yeast, employing thin-section EM and immunogold localization, suggest some interesting possibilities. Yeast Nup116 is a nucleoporin, containing GLFG repeats ([30**]; see [5] for review, and below), that localizes, in thin sections, to a position that is consistent with, or near to, the nucleoplasmic ring [30**]. Its gene is not essential for growth at 23°C, but invaginations of the INM are observed in null mutants [30**], suggesting

that the INM has been destabilized. When these null mutants are grown at 37°C, mRNA export is blocked and NE structure is severely altered. In some profiles, the ONM appears to be detached from the NPC and forms a continuous sheet over it, suggesting that the ONM is no longer constrained to be routed into the NPC, as discussed above for the function of the cytoplasmic ring. In other profiles, the INM also appears to be routed via the NPC over its cytoplasmic face, forming a little bubble or herniation over the NPC, suggesting that the INM is also not routed properly when Nup116 is missing. Thus, Nup116 could be involved somehow in maintaining the structure of one or both rings, that might function to properly route the membranes into the pore.

'Transporter'

Active transport has been shown to occur through the central channel of the NPC [31,32] (Fig. 5). As EM of thin sections and isolated NEs show variation in the material found in the central channel, some models omit a central structure. Indeed, often little is seen in this region [21]. In detergent-extracted, frozen hydrated NEs, however, most NPCs have a consistent structure that, in 3D reconstructions, appears to be an hourglass-shaped cylinder [17**]. The existence of a central structure, which is controversial [4], is supported by stereo FEISEM observation of proteolysed NEs ([8**], MW Goldberg,

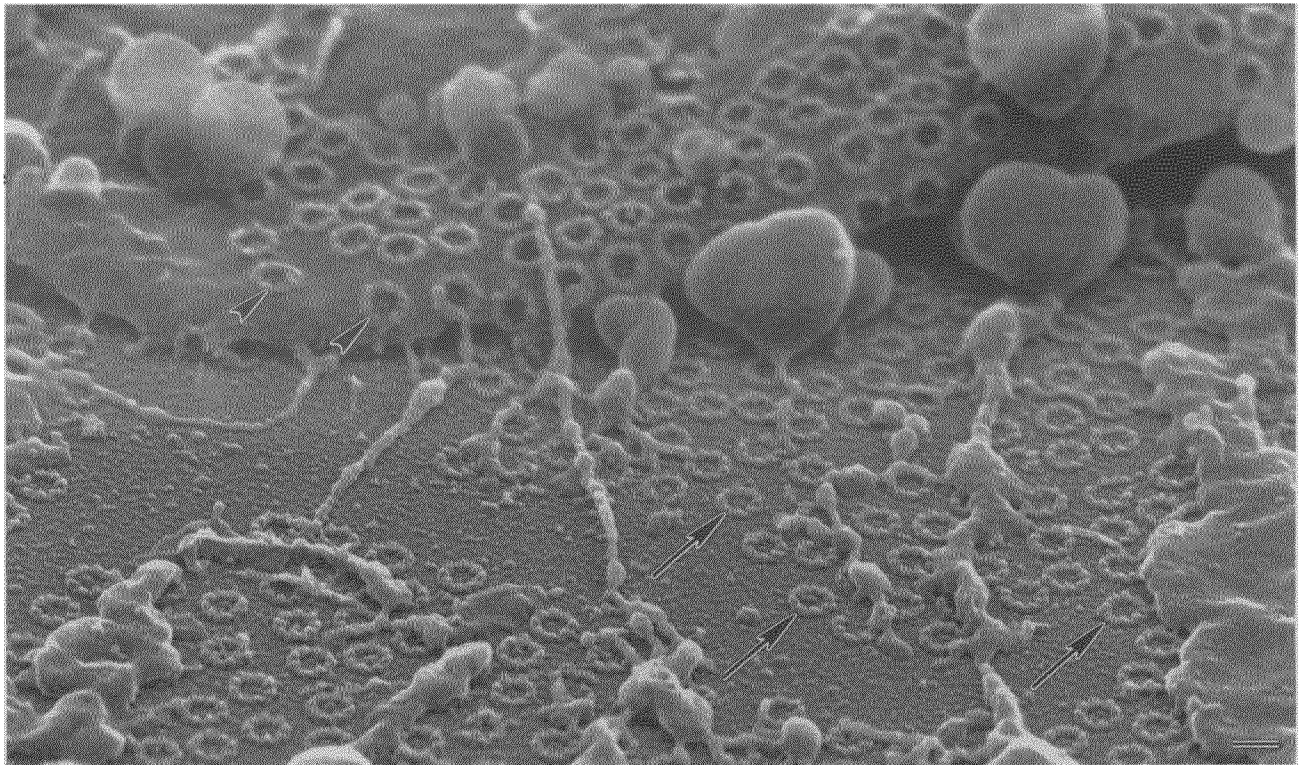


Fig. 3. A tilted FEISEM view of a *Xenopus* oocyte NE that has been rolled across the substratum to detach cytoplasmic rings. Detached rings are indicated by arrows, membrane-attached rings are indicated by arrowheads. Bar 100 nm.

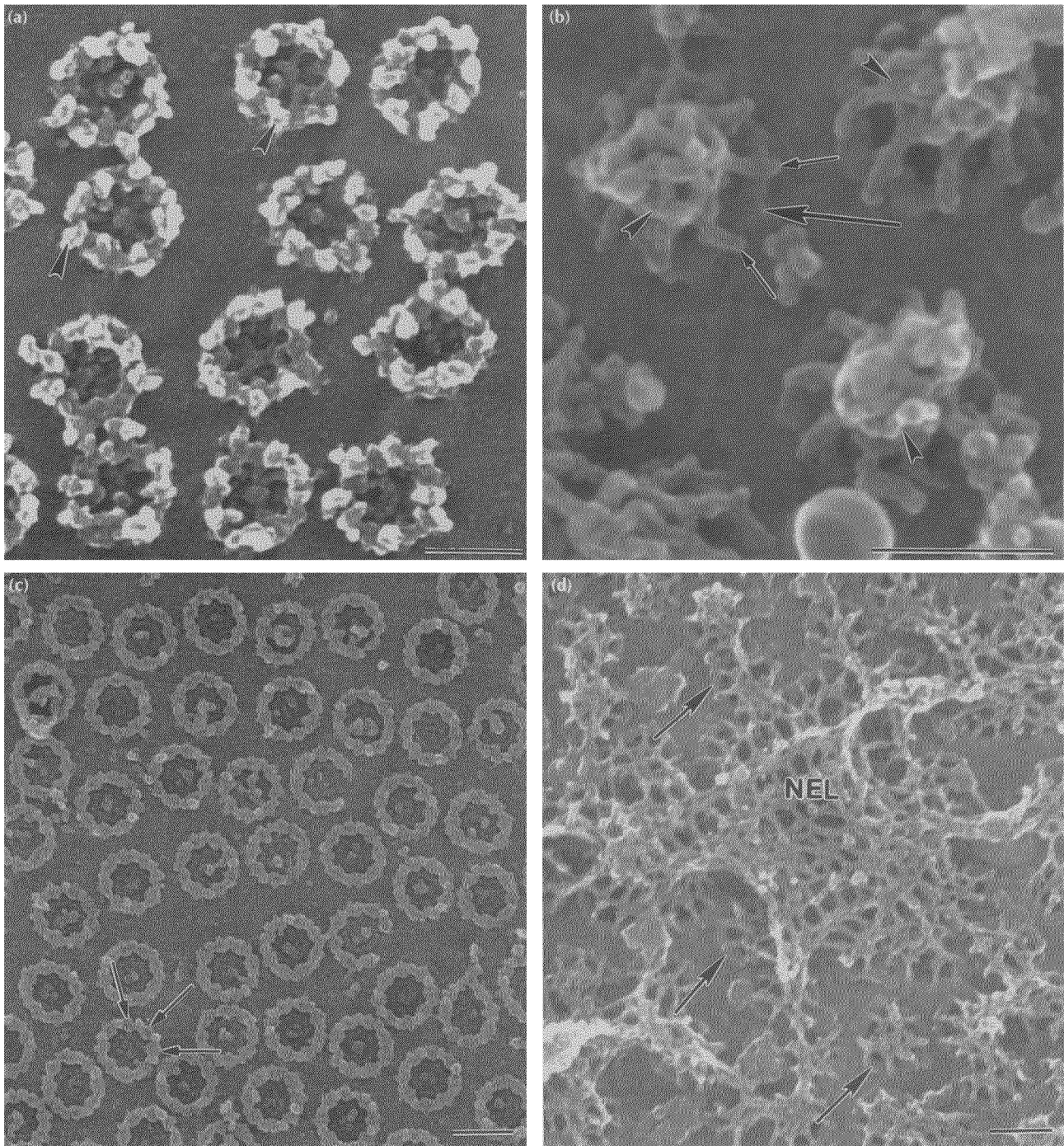


Fig. 4. FESEM of different views of amphibian oocyte NE. (a) Cytoplasmic face of intact NPCs; arrowheads indicate cytoplasmic particles. (b) High-magnification image of the nucleoplasmic face of the NPC showing basket filaments (small arrows) and the nucleoplasmic ring (large arrows). The distal basket ring is indicated by arrowheads and has an irregular appearance due to remnants of attached NEL (see [d]). (c) Cytoplasmic face of the NE after proteolysis to remove cytoplasmic particles, revealing the ring structure; subunits of the ring are indicated by the small arrows. (d) Nucleoplasmic face of a *Triturus* oocyte NE showing the NEL attached to the NPC baskets (arrows). Bars 100 nm.

unpublished data) where a consistent central structure is always observed and the channel never appears empty. It may be that this structure is not always visible, either due to a lack of contrast [17••], or to lack of access to metal coating [21]. The protein composition of the transporter is not known. Wheat germ agglutinin, which labels a

family of nucleoporins containing N-acetylglucosamine, and an antibody to the nucleoporin p62 label one or both faces of the central channel [27••], and both inhibit transport [33]. Nothing, as yet, labels within the actual channel, however, and it is uncertain whether these labels are specific to the transporter anyway.

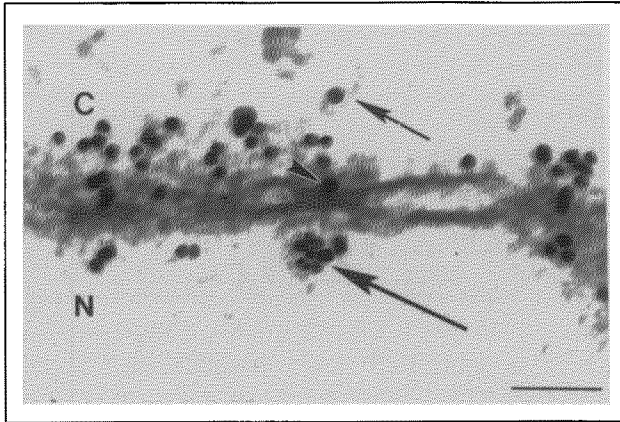


Fig. 5. Thin section of a *Xenopus* oocyte microinjected with nucleoplasm-coated gold particles showing attachment of a protein being imported to a cytoplasmic filament (small arrow) and gold particles sometimes accumulating on the nucleoplasmic side (large arrow) and movement through the central channel (arrow-head). Bar 100 nm.

Filaments of the NE

Cytoplasmic filaments

Particles [25] and/or filaments [8•,21,34,35] are attached to the subunits of the cytoplasmic ring (Fig. 4a) and may extend some distance into the cytoplasm [36]. Imported proteins may initially bind to these filaments [36] (Fig. 5) before moving to the central channel of the NPC. It is possible that they extend deep into the cytoplasm or are attached to cytoskeletal fibres. It is also clear that intermediate filaments anchor to the NE [37]. Nup180, already mentioned, could form part of these filaments and has been suggested to be the same protein as Tpr (translocated promoter region), a large coiled-coil protein that is likely to be a filament, that localizes to the cytoplasmic face of the NPC [38,39] and is involved in inactivation of oncogenic kinases. Another nucleoporin, Nup214, which also has a putative coiled-coil structure, and is localized to the cytoplasmic filaments [39,40], is homologous to CAN, a putative oncoprotein [40]. The role of these proteins in NPC function is unknown, but their presence suggests that the NPCs themselves may play a fundamental role in controlling cell activity, rather than simply being conduits into the nucleus.

Baskets

Between each subunit of the nucleoplasmic ring, attached to its outer edge [8•], are filaments of about 10 nm diameter [34] (eight per ring), which extend 30–70 nm into the nucleoplasm where they join to form a ring. This makes a basket- or fishtrap-like structure (Fig. 4b) [8•,21,34,35]. The filament structure is similar to intermediate filaments, but its protein composition has not been determined. Antibodies to Nup153 bind to the basket ring [39], but not the filaments

themselves. Nup153 contains zinc finger motifs and binds DNA in a zinc-dependent manner *in vitro* [41]. Whether it binds DNA *in vivo* is not known but this observation suggests [41] that the NPCs might organize DNA in a way that is consistent with the 'genegating' hypothesis [42]; this proposes that active genes are physically connected to specific NPCs, controlling movement of macromolecules to and from genes, and hence the activity of the genes.

Superficially, the basket looks like a sieve. Its function may be to prevent the NPC becoming clogged with the dense nuclear contents, or it may prevent ribonucleoprotein particles from entering the NPC until they have been properly processed. Alternatively, it has been suggested [21] that the basket is a dynamic structure that can be 'opened' by depleting divalent cations and reformed by adding them back. The significance of this is unknown, especially as unfolding only occurs when monovalent cations are also depleted to unphysiologically low levels (MW Goldberg, unpublished data).

Nuclear envelope lattice

A monoclonal antibody to a ~190 kDa (by SDS-PAGE) protein, thought to be Nup153, also labels extensive arrays of intranuclear filaments in amphibian oocytes [43•], which appear, in thin sections, to attach to the baskets and extend into the nucleus [44]. We believe that these filaments are the same as the recently described NE lattice (NEL) (Fig. 4d), a highly symmetrical, interwoven network attached to the baskets [35]. Presently, the NEL has been observed only in amphibian oocytes, although thin sections of yeast nuclei indicate connections between putative baskets [45]. The filaments of the NEL are similar to basket filaments so could represent a storage of basket components or a way of increasing the functional capacity of the baskets.

Connecting the NPC to nucleoplasmic structures

Recently, ultrastructural analysis of yeast cells containing nucleoporin mutations has revealed some fascinating effects on nuclear and NE morphology, some of which are discussed above.

Nucleoporins involved in RNA export

Several nucleoporins have now been shown to be involved in RNA export as well as playing structural roles in maintaining proper NE morphology. Nup116, already mentioned, is related to Nup100 and Nup145 [46]; they all belong to the family of nucleoporins that contain 'GLFG' repeats (single-letter code for amino acids), the significance of which are not known [5], and share a sequence dubbed the 'nucleoporin RNA-binding motif' [46], which may be involved in RNA export. Nup145

is required for RNA export and a deletion/disruption in the amino-terminal region results in both herniations of the nuclear membranes over the NPCs (similar to those seen in Nup116 null mutants) and NPC clustering, resulting in the formation of grape-like lobules of the NE [47**]. Deletion of Nup133, a nucleoporin also involved in RNA export, also results in NPC clustering [48**]. Clustering could result if NPCs were released from the lamina, or if other potential anchoring structures such as the basket or NEL were disrupted. Although none of the lamina, baskets or NEL have been definitely identified in yeast, thin sections of isolated yeast nuclei show evidence for the existence of these structures [45].

Structural nucleoporins

Temperature-sensitive mutations in Nup1 lead to transport defects at 37°C and multinucleate cells at all temperatures, with the NE forming long finger-like projections through the cytoplasm [49**]. This suggests that the NE has become unstable and is unable to maintain proper nuclear morphology. It was suggested [49**] that the Nup1 protein may be required to maintain structural connections between the NE and the underlying nuclear scaffold, connections that have been shown to occur [37]. Bearing in mind that Nup1 is an NPC protein, these connections may be via basket filaments or the NEL. It was also shown that Nup1 and Nup2 interact with Srp1 [50**], a protein that localizes to the NE and shows sequence similarities to β -catenin/armadillo/plakoglobin. This family of proteins interacts with the cytoplasmic domain of cadherins and may link them to the cytoskeleton. Srp1 may be involved, with Nup1 and Nup2, in an equivalent role, linking nucleoskeletal elements to the NE. Interestingly, in yeast, the nucleolus is closely associated to the NE, but when Srp1 is deleted the nucleolus becomes fragmented into the nuclear interior (see Mélése and Xue, this issue, pp 319–324) [51]. It was shown, in amphibian oocytes, that the filaments labelled by the anti-Nup153 antibody (which may be the NEL) connect between the NE and the nucleoli [43**]. If Srp1 is involved in NEL or basket integrity, its deletion would be expected to result in release of nucleoli from the NE, as is observed. Srp1 is also 44% homologous to the recently identified import factor, importin, that is necessary for the first step (binding) of nuclear import [52]. The significance of this observation has not yet been determined.

Conclusion

Ultimately, research on the NE aims to determine its structure at high resolution, identify every component and define what role each component plays. The use of various EM techniques has advanced our understanding of structures of the NE to a point where roles can be suggested for various components. Immunogold labelling

studies at the EM level are beginning to assign proteins, particularly to peripheral structures. Meanwhile, yeast geneticists are identifying novel nucleoporins at a rapid rate, with structural studies of their mutants revealing much about function and location, but being limited by the resolution of thin sections. Hence, development of methods to study yeast NE structure at relatively high resolution (as is possible with amphibian oocytes, and mammalian cells using FEISEM [53]) would be a great advance. Equally important, may be the use of cell free systems where 'mutant' NEs can be assembled [54], the cytosol can be manipulated and NE structure can be assessed by transmission EM and FEISEM [55].

Acknowledgements

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An autoimmune antibody that labels the cytoplasmic face of the nuclear pore complex was found in a human patient with overlap connective tissue disease. It identifies a protein named Nup180, a peripheral membrane protein that is extractable by 2M urea and does not react with wheat germ agglutinin (WGA). The pattern of labelling is different from WGA and anti-p62, consistent with labelling of the cytoplasmic ring or cytoplasmic filaments. The antibody does not affect transport.

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