The nuclear envelope (NE) is the double membrane structure that controls the access of molecules to and from the nucleus (Franke et al., 1981; Gerace, 1986; Newport and Forbes, 1987; Gerace and Burke, 1988). The outer nuclear membrane (ONM) is decorated with ribosomes and is continuous with the rough endoplasmic reticulum. It is joined to the inner nuclear membrane (INM) at the nuclear pores and separated from it by the perinuclear cisterna. The nuclear lamina is a network of intermediate filament lamin proteins (Aebi et al., 1986; Akey, 1989; McKeon et al., 1986; Fisher et al., 1986; Franke, 1987) that line the INM (Gerace, 1986), probably performing a structural role (Gerace, 1986), and may interact with the INM, the chromatin and the NPCs.

The NE appears to be freely permeable to small molecules and some small macromolecules and controls the passage of proteins and RNA into and out of the nucleus (Gerace and Burke, 1988; Garcia-Bustos et al., 1991; Silver, 1991). Although passive diffusion of molecules up to 40-60 kDa and 9 nm diameter occurs (Peters, 1986), small nucleophilic proteins such as histone H1 (21 kDa) are actively transported (Breeuwer and Goldfarb, 1990). Active nuclear import has two steps: binding of nucleophilic proteins, which contain a nuclear localisation signal (Garcia-Bustos et al., 1991; Silver, 1991), to a receptor on the cytoplasmic side of the NPC in an ATP-independent manner, followed by ATP-dependent translocation (Newmeyer and Forbes, 1988; Richardson et al., 1988). Active transport of gold particles coated with a nucleophilic protein is limited to a gold particle size of up to about 23 nm (Feldherr and Akin, 1991) and is inhibited by wheat germ agglutinin (Yoneda et al., 1987; Dabauvalle et al., 1988a), which binds to a subset of O-linked N-acetylglucosamine-containing nucleoporins (Hart et al., 1989) and by antibodies to these proteins (Dabauvalle et al., 1988b), which have been used to identify and localise these glycoproteins. (Snow et al., 1987). The lectin concanavalin A binds to a further NPC protein, gp210, which has been localised to the periphery of the NPC and may act as an anchor for NPCs to the NE (Gerace et al., 1982).

### INTRODUCTION

The nuclear envelope (NE) is the double membrane structure that controls the access of molecules to and from the nucleus (Franke et al., 1981; Gerace, 1986; Newport and Forbes, 1987; Gerace and Burke, 1988). The outer nuclear membrane (ONM) is decorated with ribosomes and is continuous with the rough endoplasmic reticulum. It is joined to the inner nuclear membrane (INM) at the nuclear pores and separated from it by the perinuclear cisterna. The nuclear lamina is a network of intermediate filament lamin proteins (Aebi et al., 1986; Akey, 1989; McKeon et al., 1986; Fisher et al., 1986; Franke, 1987) that line the INM (Gerace, 1986), probably performing a structural role (Gerace, 1986), and may interact with the INM, the chromatin and the NPCs.

The NE appears to be freely permeable to small molecules and some small macromolecules and controls the passage of proteins and RNA into and out of the nucleus (Gerace and Burke, 1988; Garcia-Bustos et al., 1991; Silver, 1991). Although passive diffusion of molecules up to 40-60 kDa and 9 nm diameter occurs (Peters, 1986), small nucleophilic proteins such as histone H1 (21 kDa) are actively transported (Breeuwer and Goldfarb, 1990). Active nuclear import has two steps: binding of nucleophilic proteins, which contain a nuclear localisation signal (Garcia-Bustos et al., 1991; Silver, 1991), to a receptor on the cytoplasmic side of the NPC in an ATP-independent manner, followed by ATP-dependent translocation (Newmeyer and Forbes, 1988; Richardson et al., 1988). Active transport of gold particles coated with a nucleophilic protein is limited to a gold particle size of up to about 23 nm (Feldherr and Akin, 1991) and is inhibited by wheat germ agglutinin (Yoneda et al., 1987; Dabauvalle et al., 1988a), which binds to a subset of O-linked N-acetylglucosamine-containing nucleoporins (Hart et al., 1989) and by antibodies to these proteins (Dabauvalle et al., 1988b), which have been used to identify and localise these glycoproteins. (Snow et al., 1987). The lectin concanavalin A binds to a further NPC protein, gp210, which has been localised to the periphery of the NPC and may act as an anchor for NPCs to the NE (Gerace et al., 1982).

### SUMMARY

The structure of the nuclear pore complex (NPC) has been previously studied by many different electron microscopic techniques. Recently, scanning electron microscopes have been developed that can visualise biologically relevant structural detail at the same level of resolution as transmission electron microscopes and have been used to study NPC structure. We have used such an instrument to visualise directly the structure of both cytoplasmic and nucleoplasmic surfaces of the NPC of manually isolated amphibian oocyte envelopes that have been spread, fixed, critical point dried and coated with a thin fine-grained film of chromium or tantalum. We present images that directly show features of the NPC that are visible at each surface, including coaxial rings, cytoplasmic particles, plug/spoke complexes and the nucleoplasmic basket or fishtrap. Some cytoplasmic particles are rod-shaped or possibly “T”-shaped, can be quite long structures extending into the cytoplasm and may be joined to the coaxial ring at a position between each subunit. Both coaxial rings, which are proud of the membranes, can be exposed by light proteolytic digestion, revealing eight equal subunits each of which may be bipartite. We have determined that the nucleoplasmic filaments that make up the baskets are attached to the outer periphery of the coaxial ring at a position between each of its subunits. These filaments extend into the nucleoplasm and insert at the distal end to the smaller basket ring. The space left between adjacent basket filaments would exclude particles bigger than about 25 nm, which is consistent with the exclusion limit previously found for NPC-transported molecules.

Key words: nuclear pore complex, nuclear envelope, germinal vesicle, field emission scanning electron microscopy
To understand the transport mechanism it is essential to determine the structure, composition and function of each component of the NPC and how they interact with structures such as the lamina, chromatin, membranes and cytoplasmic structures. In the current morphological models (Unwin and Milligan, 1982; Akey, 1989; Reichelt et al., 1990), the NPC consists of two coaxial rings at the ONM and INM. Between the two rings are eight spokes with vertical supports connecting these to each of the rings. A central plug or transporter may be present in the central channel. Radial arms are at the NPC periphery and are involved in anchoring the NPC in the membrane (Akey, 1989). It has recently been shown by field emission, in-lens scanning electron microscopy (FEISEM; previously referred to as high resolution SEM or HRSEM) (Ris, 1989, 1991; Goldberg and Allen, 1992) and metal-shadowed preparations in the TEM (Jarnik and Aebi, 1991) that there is a basket-like structure protruding into the nucleoplasm on the cytoplasmic face of the NPC. The complete NPC (not including peripheral structures) has a molecular mass of about 125,000 kDa (Reichelt et al., 1990) and may consist of over a hundred different proteins (Gerace and Burke, 1988).

The NE of amphibian oocyte germinal vesicles can be manually isolated to give many square micrometres of both the cytoplasmic and nucleoplasmic faces of the NE, and has been used in many electron microscopic studies of NPC and lumina structure (e.g. see Unwin and Milligan, 1982; Aebi et al., 1986; Stewart and Whytock, 1988; Akey, 1989; Reichelt et al., 1990). We have used such material to investigate NPC structure with FEISEM.

FEISEM has been used, at a resolution of ~1.0 nm, to visualise surface structure such as details of phages, Fab fragments and 1 nm colloidal gold (Herman et al., 1991; Muller and Herman, 1990; as well as chromatin (Inaga et al., 1990; Allen et al., 1986) and NE structure (Ris, 1989, 1991; Goldberg and Allen, 1992; Goldberg et al., 1992).

**MATERIALS AND METHODS**

**Preparation of germinal vesicle NE for electron microscopy**

*Triturus or Xenopus* were anaesthetized in 0.1% MS222 (ethyl m-aminobenzoate methane sulphonate, Sigma Chemical Co., St. Louis, MO), and ovaries were removed and placed on ice. Pieces of ovary were placed in 5:1 buffer (83 mM KCl, 17 mM NaCl, 10 mM Hepes, pH 7.4), pierced with a sharpened needle and the contents extruded. The germinal vesicle was picked up with a narrow bore pipette, cleaned by gently sucking up and down, and deposited on a silicon chip, where it was left to swell for a 1-5 minutes for “clean” preparations, spread with fine glass needles and washed for 20-60 minutes before fixation. Alternatively, for “rapid isolation” preparations vesicles were immediately spread and fixed with minimum swelling and washing. Some samples were incubated for 5 minutes in 1, 10 or 100 µg/ml trypsin (Worthington Biochemical Corp., Freehold, NJ) in 5:1 buffer or for one hour in 100 µg/ml DNase I (type II, which affected NPC structure, or type DN-EP, which did not, Sigma Chemical Co., St Louis, MO). Alternatively, they were treated for one hour with 90 units/ml or 180 units/ml of RNase A (Molecular Biology grade, preboiled, Sigma Chemical Co., St Louis, MO). Detergent extraction was performed for 5 minutes in 5:1 buffer + 0.5% Triton X-100 (Sigma Chemical Co., St Louis, MO). Samples were fixed in 2% glutaraldehyde, 0.2% tannic acid in 0.1 M Hepes, pH 7.4, then 0.1% OsO₄ and 1% uranyl acetate for 10 minutes each. Samples were dehydrated through an ethanol series and critical point dried from CO₂ via Arklone (ICI, Runcorn, Cheshire, U.K.) as a transitional fluid; 100% ethanol and Arklone were sometimes dried with molecular sieve and high-purity CO₂ passed through a Tousimis water filter (Tousimis Research Corporation, Rockville, MD). They were sputter-coated with a thin film of chromium or tantalum, measured to be 1.5 nm on a film thickness monitor in an Edwards Auto 306 cryopumped vacuum system (Edwards High Vacuum International, Crawley, West Sussex, U.K.) and viewed in a Topcon DS 130F field emission scanning electron microscope (Topcon Corporation, Tokyo, Japan) at 30 kV accelerating voltage.

**RESULTS**

**The cytoplasmic surface**

We have isolated oocyte NEs from the newt *Triturus cristatus* and from *Xenopus laevis*. They were spread on silicon chips, fixed, critical point dried, coated with tantalum or chromium and viewed in the top stage of a field emission SEM as previously described (Goldberg and Allen, 1992).

Fig. 1A and B shows the cytoplasmic surface of “clean” *Xenopus and Triturus* NEs, respectively. Features visible in both micrographs are: the ONM; cytoplasmic coaxial ring of the NPCs; plug-spoke complex; particles associated with the coaxial ring.

The plug-spoke complex is in general consistent with previous data, although somewhat variable, particularly in *Triturus*. The spokes radiate from the central plug (Fig. 1A) and connect to the edge of the coaxial ring. The central plug sometimes appears as a smooth disc in the central channel of the NPC and appears to be at roughly the same level as the spokes. Sometimes material appears to protrude out of the NPC, which may represent material passing through it.

The cytoplasmic coaxial ring is decorated with particles. The ring can be visualised between the particles, but is better seen when there are less than the usual eight particles present (Fig. 1A and B) or when the particles appear to have collapsed (Fig. 1A). The coaxial ring appears to have a subunit appearance (see below) in agreement with previous results (Unwin and Milligan, 1982). It has an external diameter of about 120 nm and internal diameter of 60-70 nm and possibly up to 80 nm, although the internal diameter is difficult to measure in intact NPCs. The particles are possibly positioned near the junction between two subunits (Fig. 1A and B).

When viewed from above, the particles are somewhat variable in shape and range in diameter between about 15 nm and 25 nm. When they have collapsed onto the ONM (Fig. 1A), or the NPCs are viewed at a 30° tilt (Fig. 1C) or in profile on a highly curved region (inset Fig. 1C), some of them appear as rod-shaped or filamentous particles, in agreement with previous results (Ris, 1989, 1991; Jarnik and Aebi, 1991). These rod-shaped particles are generally about 15 nm in diameter and about 30 nm long. Tilted specimens (Fig. 1C) show that not all particles are rod-shaped and some may be globular and about 25 nm in diameter,
Fig. 1. Cytoplasmic surface of “clean” oocyte NEs of *Xenopus* (A) or *Triturus* (B and C) viewed at 0° tilt (A and B), 30° tilt (C) or on a highly curved region (inset C). Features indicated are: spokes (arrow marked s); plugs (p); material protruding from central channel (m); coaxial rings (c); 15 nm particles, which are rod-shaped in tilted specimens or when collapsed (arrow); globular 25 nm particles (large arrow). Arrowheads indicate where wedge-shaped particles appear positioned between two subunits of the coaxial ring. Bars, 100 nm.
which is the same diameter as some particles on untilted specimens (Fig. 1B). Some particles appear more complex than a simple rod or sphere and are sometimes consistent with the wedge shape previously reported (Unwin and Milligan, 1982). In horizontal specimens some particles appear as 25 nm particles with a 15 nm particle on top (Fig. 1A and B), which suggests a T-shaped particle. Whether this variability is real or due to, for instance, collapse of the particles, is uncertain.

We have previously shown that rapid isolation of NEs
results in greater retention of fibrous structures attached to the nucleoplasmic face of the NPCs (Goldberg and Allen, 1992). Likewise, on the cytoplasmic face the filamentous nature of the particles that decorate the ring is more apparent, particularly in stereo pairs (Fig. 2A). The T-shape of these particles is sometimes evident with the bridge of the “T” apparently moulded around the width of the ring. The ring appears to be proud of the ONM. Other fibres are also present that are consistent with so-called pore-connecting fibres (Fig. 2B). These could simply represent filamentous particles that have collapsed onto the membrane. However, it has previously been shown that pore-connecting fibres are fairly resistant to trypsin digestion (Whytock et al., 1990) whereas it has been shown here that the particles are easily removed by proteolytic digestion (see below).

**Proteolytic digest of cytoplasmic face**

Figs 3 and 4 show the results of trypsin digestion of NEs from *Triturus* and *Xenopus*, respectively. The results, which show a sequential removal of structures, are similar for the two species except that *Xenopus* NPCs appear somewhat more sensitive to digestion. All digests were carried out for 5 minutes with 1, 10 or 100 µg/ml trypsin; 1 µg/ml trypsin removed the cytoplasmic filaments, revealing the coaxial ring of both species. However, in *Xenopus* subunits of the coaxial ring were also sometimes removed (Fig. 4A-D),
Fig. 4. Trypsin digest of cytoplasmic face of *Xenopus* NEs. (A-C) Stereo pairs of NEs digested with 1.0 µg/ml trypsin, arrows indicate central plug/transporter, arrowheads indicate spoke ring. In (C) arrowheads show the outer extent of the spoke ring. (D) shows a curved region digested with 1.0 µg/ml trypsin, arrow indicates profile of coaxial ring. (E) shows 10 µg/ml digest. Bars, 100 nm.
whereas in *Triturus* the coaxial ring appeared mostly intact even at 10 µg/ml (Fig. 3A). The coaxial rings have an external diameter of about 110 nm and an internal diameter of either 60 nm or 70 nm, depending of which part of the ring is measured. They consist of eight roughly equal subunits (as indicated in Fig. 3A) that each have a bipartite structure. The coaxial ring can be seen to be proud of the ONM in stereo pairs (Figs 2A and 4A-C) and on curved regions (Figs 3A and 4D) its thickness of 15 nm can be measured.

Within the bounds of the coaxial ring, remnants of the spoke rings and central plug/transporter are evident (Figs 3A and 4A-D). When the coaxial ring has been partially removed the extents of the spoke ring can be determined (as indicated in Fig. 4C) and its diameter measured as about 60-70 nm, similar to the internal diameter of the coaxial ring. Complete removal of the coaxial ring reveals underneath a very faintly contrasting ring, which in places can be seen pulling away from the membrane (Fig. 3B). This ring is not so evident in *Xenopus* but is presumed to be the vertical supports, previously described (Akey, 1989).

Interestingly, digestion of the coaxial ring results in a sequential shortening of the circumference of the ring rather than fragmentation (Fig. 4A-C). In other words, it is only seen as a complete ring or reduced to a horse-shoe shape, a semicircle or smaller, but never as several fragments of the same ring arranged around the pore. This suggests that on any one coaxial ring, digestion will only start at one point and work its way around the ring in one or two directions. This may mean that there is a unique trypsin-sensitive point on the coaxial ring where the enzyme is able to gain initial access to the structure.

**Nuclease digestion**

NEs were treated with preboiled RNase A and DNase I. In agreement with other results, RNase did not have any striking affect on NPC structure (Jarnik and Aebi, 1991), although preparations appeared particularly clean after such a treatment (not shown). Highly purified DNase I also had little effect, although a less-pure grade (see Materials and Methods), which presumably contained contaminating proteases, had a similar effect to trypsin (Fig. 3C). However, at a particular concentration the central plug was specifically removed without removing the spoke ring, which was not a stage observed with trypsin. The significance of this is unknown but it may suggest that different proteolytic enzymes have a different sequence of removal of structures.

**Nucleoplasmic face**

The presence of “baskets” (also called “cages” or “fish-traps”) on the nucleoplasmic face of the NPCs has previously been demonstrated by low-voltage FEISEM (Ris, 1989, 1991) metal shadowing in the TEM (Jarnik and Aebi, 1991) and FEISEM (Goldberg and Allen, 1992). Here these results are confirmed in the newt *Triturus cristatus* as well as in *Xenopus* and the structure is described.

Fig. 5A and B shows the nucleoplasmic surface of clean *Xenopus* and *Triturus* NEs, respectively. The inset in Fig. 5B shows a particularly clean *Triturus* basket. Features visible are: the INM surface; nucleoplasmic coaxial ring of the NPCs; basket filaments; distal basket rings.

The coaxial rings have an external diameter of approximately 40-45 nm in length when viewed on curved regions and tilted specimens (Fig. 5B, C and inset in C). They have a width of about 10 nm (Fig. 5) and appear to attach to the outer periphery of the coaxial ring (Fig. 5C) at the junction between two of its subunits (inset Fig. 5B). They extend into the nucleoplasm, where each attaches to a distal basket ring. The distance between adjacent filaments is about 25-30 nm at the coaxial ring and 10-15 nm at the basket ring. The basket ring may be a ring (external diameter of ~65-70 nm, internal diameter of ~20-30 nm) or a flat disc or may be decorated with a varying amount of material. Basket rings may be interconnected by fibres (Jarnik and Aebi, 1991; Goldberg and Allen, 1992) or by the previously described NE lattice (NEL) network (Figs 5C, 7A and C). The inset in Fig. 5A illustrates the fact that although the number of basket filaments that can be counted number almost invariably up to eight they occasionally have nine.

**Detergent treatment**

Treatment of *Triturus* NEs with the non-ionic detergent Triton X-100 (Fig. 6) removes membrane components, leaving apparently intact NPCs joined together at the nucleoplasmic coaxial ring by fibres that are presumed to be the lamina. Remnant NEL is present that is clearly at a different level to the putative lamina and is attached to the basket rings. Putative lamin fibres are variable in diameter and may be as small as 7-8 nm. Results with *Xenopus* are similar except they generally have little NEL or even remnants of NEL.

**Proteolytic digest of nucleoplasmic face**

Figs 7 and 8 show the results of trypsin digests of the nucleoplasmic side of NEs from *Triturus* or *Xenopus*, respectively. Again, the results are similar in the two species, except *Xenopus* appears to be more sensitive: 10 µg/ml trypsin completely removes nucleoplasmic coaxial rings in *Xenopus* (Fig. 8E-H) and only partially removes some in *Triturus* (Fig. 7B). Compared to cytoplasmic coaxial rings (Figs 3 and 4), both *Xenopus* and *Triturus* nucleoplasmic coaxial rings are more sensitive to trypsin, with 1 µg/ml removing some subunits in *Triturus* (Fig. 7C) and sometimes the whole ring in *Xenopus* (Fig. 8D).

Like the cytoplasmic filaments, the basket filaments are readily removed by trypsin (Figs 7 and 8) but the NEL appears to be more resistant (Fig. 7A and C), even in *Xenopus* (Fig. 8A), although its presence presumably depends on the presence of some basket filaments.

Successive degrees of coaxial ring disassembly are displayed in stereo pairs (Fig. 8B-D). There are intact rings (Fig. 8B), which appear somewhat thinner than cytoplasmic rings (Fig. 4), rings with one or two subunits removed (Fig. 8B), rings with only one or two subunits remaining (Fig. 8C) and NPCs with the ring completely removed (Fig. 8D). Like the cytoplasmic coaxial rings, we rarely observe fragmented rings, suggesting that these too are progressively digested around the ring from a point. Again the spoke ring and plug/transporter become clear and their
Fig. 5. Nucleoplasmic face of oocyte NEs from *Xenopus* (A) or *Triturus* (B and C) viewed at 0° tilt (A and B) or 30° tilt (C) or on highly curved region (inset C). Subunits of the coaxial ring are shown (inset B, arrowheads) and the connection of basket filaments to the outer periphery of the coaxial ring between these subunits is indicated (arrow in C and inset B). The inset in (C) shows the height of the basket (arrows). In *Triturus* (B and C) NEL (nel) is present and remnant NEL is present in *Xenopus* (A). Other fibres (arrowheads) in (A) are probably actin filaments (Jarnik and Aebi, 1991). A basket with 9 filaments (1-9) is shown in the inset in (A). Bars, 100 nm.
dimensions are approximately the same as measured from the cytoplasmic side.

Trypsin at 10 µg/ml removes some plugs in *Triturus*; however, in *Xenopus* the coaxial rings are completely removed (Fig. 8E-H), as are the plugs and sometimes spoke rings (Fig. 8E). Sometimes, putative vertical supports are removed without the removal of the cytoplasmic coaxial ring, which can be seen through the hole left by removal of the rest of the structure (Fig. 8F and G). This is not consistent with results seen from the cytoplasmic face where the cytoplasmic coaxial ring is digested prior to internal structures. This is probably due to the cytoplasmic ring being protected from the enzyme by its orientation on the silicon support chip.

Finally, fine fibres are observed lying on and proud of the INM in lightly digested samples (Fig. 8B-D). These are about 7 nm in diameter and are sometimes seen as interwoven square arrays. This is consistent with the lamina, although the square repeat appears smaller than that previously reported (Aebi et al., 1986) and the distance is difficult to measure due to lack of contrast. The diameter of 7 nm is also consistent with some fibres of the putative lamins observed at this level after Triton X-100 treatment (Fig. 6). Thicker fibres observed after detergent treatment may be aggregates of these thinner fibres.

**DISCUSSION**

Models of NPC structure have been built up using data from thin sections (Franke and Scheer, 1970; Jarnik and Aebi, 1991), negative-stained air-dried preparations (Unwin and Milligan, 1982; Reichelt et al., 1990; Jarnik and Aebi, 1991), frozen-hydrated (Akey, 1989, 1990) and metal-shadowed specimens (Aebi et al., 1986; Stewart and Whytock, 1988; Whytock et al., 1990; Jarnik and Aebi, 1991). Although it has been known for many years that fibrous structures are attached to each of the coaxial rings (Franke and Scheer, 1970) it has only recently been shown by FEISEM (Ris, 1989, 1991; Goldberg and Allen, 1992) and by metal-shadowed preparations in the TEM (Jarnik and Aebi, 1991) that eight nucleoplasmic filaments form a basket or fishtrap-like structure on each NPC and that these are directly attached to a filamentous network, the NEL (Goldberg and Allen, 1992). Here, using FEISEM, many features of the NPCs have been visualised confirming previous results and providing some new information.

**Cytoplasmic filamentous particles**

In agreement with others (Ris, 1989, 1990; Jarnik and Aebi, 1991) it has been shown that some of the particles attached to the cytoplasmic coaxial ring have a filamentous appearance. Whether this is a simple 15 nm wide, 30 nm long rod or a more complex structure is uncertain. The appearance of rapidly isolated NEs suggests that labile material may be lost when NEs are washed. In addition, some collapsed particles and particles on tilted specimens have an appearance that suggests an upside-down “T” or maybe an “L”-shaped particle.

It is possible that these structures observed on isolated NEs represent remnants of larger filamentous structures that extend into the cytoplasm. The differences, particularly...
Fig. 7. Proteolytic digestion of nucleoplasmic surface of *Triturus* NE with 1.0 µg/ml (A and C) or 10 µg/ml (B) trypsin for 5 minutes. Basket filaments are readily removed. NEL (nel) appears more resistant and removal of nucleoplasmic coaxial ring is more variable than the cytoplasmic ring. Subunits of the coaxial rings are evident (arrowheads). Bars, 100 nm.
Fig. 8. Stereo pairs (A-D) of nucleoplasmic face of *Xenopus* NE digested with 1.0 µg/ml trypsin for 5 minutes or with 10 µg/ml trypsin (E-H). Basket filaments are readily removed with NEL (nel) apparently more resistant. In (A-D) arrows indicate plug/transporters, crossed arrows indicate outer extent of spoke rings and arrowheads indicate square array of putative lamina. In E-H spoke rings are shown by arrowheads and cytoplasmic coaxial rings by arrows. Bars, 100 nm.
between rapidly isolated NEs and washed NEs, may be due to differential loss of material caused by shear forces during nuclear isolation or subsequent proteolysis or disassembly of polymers. However, the apparent complexity of peripheral components of the NPC in rapidly isolated NEs (Fig. 2) can be contrasted to the simple octagonal arrangement of particles attached to the cytoplasmic coaxial ring of some well-preserved NPCs in “clean” preparations (Fig. 1). This suggests that peripheral fibres (which may be complex) are attached, possibly via peripheral particles, to eight specific regions of the coaxial ring. This may be at a position between two adjacent subunits of the coaxial ring.

**Cytoplasmic coaxial ring**

The cytoplasmic coaxial ring is difficult to visualise in intact NPCs as it is partially obscured by peripheral particles/fibres, particularly in rapidly isolated preparations. The outer diameter is measurable (110-120 nm) and generally consistent with other data (Unwin and Milligan, 1982). The inner diameter is more difficult to measure due to the presence of internal structures as well as peripheral structures that confuse the position of its edge. However, a maximum internal diameter of 70-80 nm can be measured in some NPCs, which is consistent with other studies (Akey, 1989; Hinshaw et al., 1992). Smaller internal diameters of <70 nm may represent internal structure such as spoke rings (Akey, 1989).

Digestion of NPCs with proteolytic enzymes removes peripheral structures, revealing an apparently intact coaxial ring. External and internal edges and the subunit structure become clear. The eight subunits appear as regular regions of maximum or minimum width around the ring that have an internal diameter about 55 nm at the narrowest and 70 nm at the widest. The external diameter is about 110 nm.

In curved regions and stereo pairs it is evident that the coaxial ring is proud of the ONM and has a thickness of about 15 nm.

Partial digestion of coaxial rings seems to result in a sequential removal of subunits, rather than a fragmentation of the ring. This suggests that there may be a particularly trypsin-sensitive point on the coaxial ring where the enzyme is able to gain access to the structure. It also suggests that the surface of the coaxial ring may be trypsin-resistant and the subunits can only be easily digested from their ends. It may be that the surface of the coaxial rings are protected from digestion by, for instance, glycosylation, and that the differential digestion between *Triturus* and *Xenopus* could be due to a differential degree of protection. However, it is not known whether the coaxial ring proteins are modified, or whether there is any difference in the modifications between *Xenopus* and *Triturus*. This will be investigated further.

Further digestion removes the coaxial ring and reveals a very faintly contrasted ring whose surface is at the level of the ONM. This could possibly represent remnants of the structure previously described as the vertical supports (Akey, 1989), or may be a third, internal, ring.

**Internal structure**

It is difficult to gain information on structures that are not present at the outer surface by imaging secondary emitted electrons. We have partly addressed this problem by using proteolytic enzymes to partially dismantle the NPCs, although the structures of interest are clearly affected by the enzyme activity.

The plug/spoke complex is visualised in intact NPCs. Upon digestion the spokes become a diffuse ring with the plug/transporter at the centre. These become particularly clear upon removal of either coaxial ring.

Although this approach may not provide information on unaffected fine structural detail it is a useful way to uncover internal structure that is still recognisable, and possibly make previously hidden epitopes accessible to antibodies. In particular, deeply internal structures such as the putative vertical supports, mentioned above, can be exposed and examined.

**Nucleoplasmic face**

The dominant feature of the nucleoplasmic face of critical-point-dried (Ris, 1989, 1991; Goldberg and Allen, 1992) or freeze-dried (Jarnik and Aebi, 1991) NPCs from manually isolated and spread amphibian oocyte NEs viewed at the surface is the NPC basket or fishtrap, which has also been suggested from studies of thin sections (Jarnik and Aebi, 1991). The method of sample preparation is critical for the preservation of these fragile structures. They are not generally observed in air-dried preparations by TEM (Unwin and Milligan, 1982), by SEM (M. W. Goldberg unpublished result) or even in frozen-hydrated samples (Akey, 1989), probably due to the effects of surface tension (Akey, 1991).

Here, it has been confirmed that the nucleoplasmic coaxial ring consists of eight bipartite subunits (Unwin and Milligan, 1982). It has been shown that the filaments of the baskets, which usually number up to eight, attach to the outer periphery of the coaxial ring between two adjacent subunits. These filaments appear to extend to about the level of the INM, which could allow them to interact with the membrane itself or possibly with the nuclear lamina, which is probably at this level as shown in detergent-extracted and trypsin-digested preparations (see also Stick et al., 1988; Snow et al., 1987; Ris, 1989, 1991; Jarnik and Aebi, 1991; Goldberg and Allen, 1992). The diameter of the basket filaments is consistent with that of an intermediate filament such as the nuclear lamina, although this remains to be shown.

The NPC basket has the appearance of a sieve. If it is assumed that the basket is fairly rigid, then its dimensions would exclude the passage of particles greater than about 20-25 nm in diameter. This is consistent with the size limit of particles that are able to be transported through the NPC (Feldherr and Akin, 1991). This does not necessarily mean that the baskets are the limiting structure. The channel in the central transporter is probably more limiting (Akey, 1990). However, the baskets could function as an initial excluder to prevent the NPC becoming clogged with material that is too large to be transported. Baskets could also prevent large RNP granules (such as the Balbiani ring granules) from entering the NPC until they have been transformed to the rod-shaped particle that can be transported through the NPC (Stevens and Swift, 1966). The export of RNA from the nucleus appears to involve several levels of control (Reidel and Fashold, 1992) including detachment...
from the nuclear matrix, RNA processing and association with proteins. It is possible that the NPC baskets either provide a binding site for pre-export events or they are a barrier (which may be physical or biochemical) to prevent export before these events have occurred.

Proteolytic digestion shows that the basket filaments are removed in a similar way to the cytoplasmic particles/filaments, leaving the nucleoplasmic ring, which has a similar appearance to the cytoplasmic ring. However, it may be thinner and more sensitive to proteolysis, possibly due to its suggested lower molecular mass (Reichelt et al., 1990).

Despite the easy removal of basket filaments, the NEL appears more resistant to trypsin, in both species. The putative lamina is also resistant to low concentrations of trypsin, and in fact becomes clear to see after a brief period of digestion. It appears as 7 nm filaments, in close contact with the INM, arranged in places as a square array, in agreement with previous results (Aebi et al., 1986), but possibly with a smaller repeat.

In summary, we have confirmed the filamentous nature of the cytoplasmic filaments of the NPC and shown that some have the appearance of an up-side-down “T” and appear to attach at a position between two adjacent subunits of the cytoplasmic coaxial ring. We have directly visualised the subunits of the coaxial rings and shown that they are proud of the membranes by about 15 nm and that both rings may have a unique trypsin-sensitive region. The spokes and central plug/transporter have also been directly visualised. We have shown that the nucleoplasmic filaments of the NPC baskets attached to the coaxial ring at its outer periphery at a position between two adjacent subunits of the ring. The basket has the appearance of a sieve with a “hole” size that is consistent with the largest particle size that is able to be transported through the NPC. Putative lamina has been visualised in both detergent-treated and trypsin-digested samples with an individual minimum fibre diameter of about 7-8 nm.

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binding to the nuclear envelope followed by slower translocation through the nuclear pores. Cell 52, 655-664.


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