Interactions and Three-dimensional Localization of a Group of Nuclear Pore Complex Proteins

Nelly Panté,* Ricardo Bastos,‡ Isabel McMorrow,‡ Brian Burke,‡ and Ueli Aebi*§

*M. E. Müller Institute for Microscopy, Biozentrum, University of Basel, CH-4056 Basel, Switzerland; ‡Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115; and §Department of Cell Biology and Anatomy, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Abstract. We have used antibodies directed against a number of nuclear pore complex (NPC) proteins to determine their mutual interactions and location within the three-dimensional structure of the NPC. A monoclonal antibody, termed QE5, recognized three NPC polypeptides, p250, NUP153, and p62 on Western blots, and labeled the nuclear envelope of several cultured cell lines by immunofluorescence microscopy. These three polypeptides contained O-linked *N*-acetylglucosamine residues and were released from the NPC by detergent/high-salt treatment as discrete high molecular weight complexes. p250 was found in association with a novel 75 kD protein, NUP153 was released as a homo-oligomer of about 1 megadalton, and p62 was associated with polypeptides of 58 and 54 kD (previously reported by Finlay, D. R., E. Meier, P. Bradley, J. Horecka, and D. J. Forbes. 1991. *J. Cell Biol.* 114:169–183). p75, p58, and p54 were not galactosylated in vitro. *Xenopus* oocyte NEs were labeled with gold-conjugated QE5 and prepared for electron microscopy by quick freezing/freeze drying/rotary metal shadowing. This EM preparation method enabled us to more precisely localize the epitopes of this antibody to the cytoplasmic filaments and the nuclear basket of the NPC. Since QE5 recognizes three O-linked NPC glycoproteins, its labeling was compared with that of the lectin wheat germ agglutinin which recognizes O-linked *N*-acetylglucosamine moieties. The two probes were found to yield similar, although not identical, distributions of label. To identify the individual proteins with particular NPC components, we have used an anti-peptide antibody against NUP153 and a monospecific anti-p250 polyclonal antibody. Labeling with these two antibodies has documented that NUP153 is a constituent of the nuclear basket with at least one of its epitopes residing in its terminal ring, whereas p250 is a constituent of the cytoplasmic filaments.

THE nuclear pore complex (NPC)[1] is a macromolecular assembly embedded in the double-membraned nuclear envelope (NE) that mediates bi-directional molecular trafficking between the cytoplasm and the nucleus of interphase cells. Three-dimensional (3-D) EM (Unwin and Milligan, 1982; Hinshaw et al., 1992; Akey and Radermacher, 1993), in combination with a number of specimen preparation methods and microscopy techniques (Unwin and Milligan, 1982; Akey, 1989; Reichelt et al., 1990; Jarnik and Aebi, 1991; Ris, 1991; Goldberg and Allen, 1992; Ris and Malecki, 1993; Goldie et al., 1994) have revealed the architecture of the NPC and defined its major components. Thus, a consensus model of the basic framework of the NPC consisting of eight multi-domain spokes embracing a central channel complex is slowly but definitively emerging (reviewed by Panté and Aebi, 1993, 1994). Accordingly, each spoke is built of two approximately identical halves, hence, the spoke complex yields 822 symmetry with one half-spoke as the asymmetric unit. 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" or "fishtrap" being made of eight thin, 50–100-nm long filaments joined distally by a 30–50-nm-diam terminal ring. In some species (e.g., *Triturus*), the nuclear baskets are associated with a fibrous lattice, termed the "NE lattice" or NEL, which is distinct from the nuclear lamina (Goldberg and Allen, 1992). In all likelihood, the NPCs are attached directly to the nuclear lamina, although the molecular components involved in this specific association have not yet been identified.

STEM mass analysis has revealed masses of 124 megadaltons (MD) for the intact, membrane-associated NPC, and 52

Address all correspondence to U. Aebi, M. E. Müller Institute for Microscopy, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.

1. *Abbreviations used in this paper*: GlcNac, O-linked *N*-acetylglucosamine; MD, megadaltons-10^6 daltons; MBS, Modified Barth's saline; LSB, low-salt buffer; NE, nuclear envelope; NPC, nuclear pore complex; NRK, normal rat kidney; TEA, triethanolamine; 3-D, three-dimensional; WGA, wheat germ agglutinin.

MD for the detergent-released NPC (i.e., the spoke complex; Reichelt et al., 1990). Thus the mass of one half-spoke amounts to ~3.3 MD, i.e., it is on the order of a ribosome. To date, ~20–30 NPC proteins have been identified, isolated, and characterized, and ~12 of these have been cloned and sequenced. Many of these are probably constituents of the peripheral NPC components such as the rings or filamentous structures. Thus far, the only NPC protein which has been identified as a constituent of the basic framework of the NPC is gp210, a transmembrane glycoprotein bearing N-linked high mannose oligosaccharides with most of its mass residing in the perinuclear space of the NE (Gerace et al., 1982; Wozniak et al., 1989; Greber et al., 1990; Gerace, 1992). gp210 has been proposed to be a component of the radially outermost domain of the spoke complex, i.e., the "lumenal" domain, which extends into the perinuclear space (Hinshaw et al., 1992; Akey and Radermacher, 1993) and appears as distinct "knobs" in thin sections (Jarnik and Aebi, 1991). Hence, it has been proposed that gp210 may act as a membrane anchor for the NPC (Greber et al., 1990; Gerace, 1992). Using immunological approaches, a family of 10 or more vertebrate glycoproteins have been identified as NPC proteins (Snow et al., 1987; Holt et al., 1987; Davis and Blobel, 1987). The members of this family are modified with O-linked *N*-acetylglucosamine (GlcNac) moieties, and therefore bind the lectin wheat germ agglutinin (WGA). More recently, a group of at least 30 proteins that do not contain carbohydrate moieties have been identified and suggested to be NPC proteins (Radu et al., 1993). However, only one of these—NUP155—has so far been localized to the NPC by both immunofluorescence microscopy and immuno-EM (Radu et al., 1993).

Three of the vertebrate NPC glycoproteins, p62 (Starr et al., 1990; Carmo-Fonseca et al., 1991; Cordes et al., 1991), NUP153 (Sukegawa and Blobel, 1993; McMorrow et al., 1994), and POM121 (also classified as a transmembrane protein, see below; Hallberg et al., 1993) have now been cloned and sequenced. Accordingly, these share a number of common features: they are rich in serine and threonine residues, and they harbor multiple copies of a more or less degenerate pentapeptide repeat motif, XFXFG. These repeats (up to 30 in NUP153) are clustered within each protein. In p62 these repeat motifs are found within the NH_2-terminal half of the molecule, while in NUP153 and POM121 they are located towards the COOH-terminal domain. Recently, partial amino acid sequences of the 210-kD O-linked NPC glycoprotein, originally described by Snow et al. (1987), were reported (Kraemer et al., 1994) and found to have a high degree of similarity with the amino acid sequence deduced from the cDNA sequence of human CAN, a putative oncogene product of 214 kD associated with myeloid leukemogenesis which also exhibits degenerate XFXFG pentapeptide motifs (Von Lindern et al., 1992). These repetitive XFXFG sequence motifs are considered a diagnostic feature for the O-linked NPC glycoproteins, since the deduced amino acid sequences of both the N-linked NPC glycoprotein gp210 (Wozniak et al., 1989) and that of NUP155 (Radu et al., 1993) are devoid of any such repetitive sequence motifs. To date, at least six yeast NPC proteins have been cloned and sequenced (reviewed by Dingwall, 1993), and these too contain repetitive sequence motifs. Three of these, NSP1, NUP1, and NUP2 contain numerous copies of the XFXFG pentapeptide repeat characteristic of the vertebrate WGA-binding NPC glycoproteins. The other three, NUP/NSP49, NUP100, and NUP/NSP116 contain a novel tetrapeptide repetitive motif, GLFG. It is not clear whether any of these yeast NPC proteins are glycosylated. At least protein blots of isolated yeast nuclei do not bind WGA (Davis and Fink, 1990), which could mean that the sugar residues have been removed during cell wall digestion, a step required for isolation of yeast nuclei (Hurt et al., 1988).

In addition to the repetitive XFXFG motifs, POM121 contains at its NH_2-terminal end a hydrophobic region which has been proposed to anchor the NPC via this protein to the nuclear membrane (Hallberg et al., 1993). In contrast to gp210 (see above), most of the mass of POM121 appears to be accommodated within the NPC. 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 (Cys_2-Cys_2) which at least in vitro bind DNA in a zinc-dependent manner (Sukegawa and Blobel, 1993).

While immunofluorescence microscopy and immuno-EM have been used successfully to localize these various proteins to the NPC, less has been established concerning their identification with distinct NPC components. One of the major problems with these localization studies has been the cross-reactivity of the available antibodies. For example, a polyclonal mouse anti-p62 antibody labeled both the nucleoplasmic and cytoplasmic faces of NPCs of mouse liver NEs (Cordes et al., 1991), but only the nucleoplasmic face of NPCs of *Xenopus* oocyte NEs (Cordes et al., 1991; Panté and Aebi, 1993). Using a polyclonal antibody raised against a fusion protein expressed from a NUP153 cDNA construct, NUP153 has been unequivocally localized to the nucleoplasmic face of the NPC (Sukegawa and Blobel, 1993), and more specifically to intranuclear filaments attached to the nuclear periphery of the NPC (Cordes et al., 1993). NUP180, a NPC polypeptide of 180 kD which does not bind WGA, has recently been located to the cytoplasmic ring and possibly the cytoplasmic filaments associated with it using an affinity-purified antibody from an auto-immune serum obtained from a patient with an overlap connective tissue disease (Wilken et al., 1993).

Relatively little is known concerning the mutual interactions of NPC proteins to form distinct complexes within the NPC. In vertebrate systems for instance, it was first reported that some of the soluble NPC proteins contained in in vitro nuclear reconstitution extracts form a large supramolecular complex (Dabauvalle et al., 1990). To date, only one vertebrate NPC complex has been identified and characterized at the molecular level. It is required for nuclear protein import (Finlay et al., 1991), and it contains the O-linked glycoprotein p62 interacting with at least p58 and p54 (Finlay et al., 1991; L. Gerace, personal communication). These latter two polypeptides may be glycosylated too, but if so they are to a much lesser extend than p62 (Finlay et al., 1991). In yeast, Hurt (1990) has expressed the α-helical COOH-terminal domain of NSP1 and found that the fusion protein could associate with yeast NEs. Based on this observation, he proposed that NSP1 would interact with some component(s) of the yeast NPC. More recently, NSP1 was indeed found to form a complex with NUP/NSP49 (Wimmer et al., 1992).

We have now begun to examine in detail the interactions

and location of two WGA binding NPC glycoproteins, NUP153 and p250. We find that both of these O-linked glycoproteins may be dissociated from NPCs as distinct complexes with a defined polypeptide composition, thus providing information about potential protein–protein interactions within the NPC. Furthermore, employing a variety of high-resolution immuno-EM techniques, we have been able to localize NUP153 and p250 to distinct peripheral components within the 3-D architecture of the NPC.

Materials and Methods

Cell Culture

Normal rat kidney (NRK), BHK, and HeLa cells were cultured at 37°C in D.MEM containing 10% FCS (both from Hyclone, Logan, UT) and penicillin/streptomycin (GIBCO BRL, Gaithersburg, MD). *Xenopus* laevis A6 (*X.l.* A6) cells were cultured in 75% NCTC109 medium (GIBCO BRL), 15% H_2O and 10% FCS at 26°C. All cells were grown in a humidified incubator in a 7.5% CO_2/92.5% air atmosphere.

Purification of Rat Liver Nuclear Envelopes

Nuclei were isolated from 40-g batches of rat livers according to the method of Blobel and Potter (1966). From these, salt-washed nuclear envelopes were prepared as described by Gerace et al. (1984).

Antibodies

The monoclonal antibody QE5 was produced by conventional procedures (Taggart and Samloff, 1982). In short, spleen cells from Rb(8.12) 5BnR mice immunized with rat liver nuclear envelope proteins extracted in TX buffer (50 mM triethanolamine [TEA], pH 7.4, 500 mM NaCl, 0.5% Triton X-100, 1 mM DTT, 1 mM PMSF and 1:1,000 CLAP [10 mg/ml each of chymostatin, leupeptin, antipain, and pepstatin in DMSO]) were fused with FOX NY myelomas. Hybridoma cultures were screened using a combination of immunofluorescence microscopy and immunoblotting. Where appropriate, hybridomas were cloned twice by suspension at low dilution in soft agar (Coffino and Scharff, 1971). An antibody against NUP153 was raised in guinea pigs using the synthetic peptide KTGNFKFGDQG designed on the basis of the primary sequence of human NUP153 (McMorrow et al., 1994) as immunogen following the procedure described by Kreis (1986). The NH_2-terminal lysine was added to facilitate covalent coupling to keyhole limpet hemocyanin (Calbiochem-Behring Corp., San Diego, CA) used as carrier protein. The resulting antiserum was affinity purified on a CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden) 4B column to which the synthetic peptide used as immunogen was coupled. A rabbit anti-peptide antibody directed against human lamin A has been previously described (Burke, 1990). An anti-p250 antibody was prepared by immunizing guinea pigs with 10–20 μg of p250 contained in preparative gel slices. p250 was obtained by absorbing a rat liver nuclear envelope extract in TX buffer (see above) to an affinity matrix consisting of QE5 IgG covalently coupled to protein G–Sepharose using dimethylpimelimidate (Harlow and Lane, 1988). A polyclonal antibody against preparative gel-purified NUP153 was prepared in a similar fashion.

Immunofluorescence Microscopy

Cells grown on glass coverslips were fixed with formaldehyde and labeled with antibodies as described by Ash et al. (1977). Rhodamine-conjugated secondary antibodies were obtained from Tago, Inc. (Burlingame, CA). To delineate the chromosome distribution as a reference, all samples were stained with Hoechst dye (No. 33258; Sigma Chemical Co., St. Louis, MO). Specimens were observed and photographed with a Zeiss Axiophot microscope equipped with a 63× Plan-APO (1.4 NA) objective lens.

^{35}S Labeling of Cells, Preparing Cell Extracts, and Performing Immunoprecipitations

Cells grown in 35-mm petri dishes were labeled overnight with ^{35}S-trans label (^{35}S-met and ^{35}S-cys; ICN Radiochemicals, Costa Mesa, CA) at 50 μCi/ml in met/cys-free D.MEM containing 5% FCS, and dialyzed against PBS and 10% normal D.MEM (containing 1/10 the normal concentration of unlabeled met and cys).

Labeled or unlabeled cells were washed once in PBS before lysed in TX buffer (see above) to produce TX extracts, or in a low-salt buffer (TX buffer containing 100 mM instead of 500 mM NaCl) to yield low-salt extracts. The cell extracts were obtained by centrifugation of the cell lysate for 5 min in an Eppendorf microfuge at 4°C. The pellet was discarded, and the supernatant (i.e., the cell extract) was processed as follows: 1–5 μg of primary antibody (monoclonal or guinea pig IgG) and 20 μl of a 50% suspension of protein A–Sepharose in PBS were added to the cell extracts. For QE5 immunoprecipitation, 3 μl of rabbit anti–mouse IgG serum was included as a "bridging" antibody. The extract was then slowly rotated overnight at 4°C. The next morning, the protein A–Sepharose was washed five times in either low-stringency TX buffer (see above) or high-stringency SDS/TX buffer (50 mM TEA, pH 7.4, 100 mM NaCl, 0.5% Triton X-100, 0.1% SDS, 1 mM DTT, 1 mM PMSF, and 1:1,000 CLAP). After two more washes in 50 mM Tris, pH 7.4, the protein A–Sepharose pellets were suspended in SDS-PAGE sample buffer as appropriate (Laemmli, 1970). Gels containing ^{35}S-labeled proteins were fixed in 10% trichloroacetic acid, impregnated with En^{3}Hance (New England Nuclear/Du Pont, Wilmington, DE), dried, and exposed to x-ray film (Kodak XAR) at −70°C. For some experiments, a protein G affinity matrix was substituted for the protein A Sepharose mixture. Rat liver NEs were also extracted in low-stringency TX buffer and processed for QE5 immunoprecipitation using the protein G affinity matrix.

In Vitro Galactosylation

Washed QE5 immunoprecipitates obtained from confluent BHK cells grown in 35-mm petri dishes were labeled for 30 min at 37°C with 5 μCi UDP-[^{3}H]-galactose (Amersham Buchler GmbH, Braunschweig, Germany) in the presence of 100 mU galactosyl transferase (Sigma Chemical Co.) using conditions previously described (Holt and Hart, 1986; Holt et al., 1987). The reaction was terminated by the addition of EDTA to 50 mM followed by two washes of the immunoprecipitates in 50 mM TEA, pH 7.4. Galactosylated immunoprecipitates were analyzed by SDS-PAGE followed by fluorography (Holt and Hart, 1986).

Immunoblotting

Protein mixtures separated by SDS-PAGE were transferred onto nitrocellulose filters (BA85; Schleicher & Schuell, Keene, NH) as described by Burnette (1981), using a semi-dry blotting apparatus (Hoeffer Scientific Instruments, Inc., San Francisco, CA). Filters were blocked with 5% nonfat dry milk, labeled with primary antibodies, and developed either with peroxidase-conjugated secondary antibodies as previously described (Burke et al., 1982), or with ^{125}I-labeled protein A (New England Nuclear/Du Pont).

Isolation of Xenopus Oocyte Nuclei and Preparation of Nuclear Envelopes

Mature (stage six) oocytes were surgically removed from female *Xenopus laevis* as described previously (Reichelt et al., 1990; Jarnik and Aebi, 1991), and stored in Modified Barth's saline (MBS) containing 88 mM NaCl, 1 mM KCl, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 10 mM Hepes, pH 7.5, and 100 U/ml of penicillin-streptomycin. Individual oocytes were placed in a small petri dish filled with low-salt buffer (LSB) containing 1 mM KCl, 0.5 mM $MgCl_2$, 10 mM Hepes, pH 7.5. The nuclei were manually isolated by opening the oocytes at their vegetal pole with fine forceps. The popped out nuclei were cleaned of yolk and other material by sucking them up and down in a 5-μl pipette in LSB. For bulk preparation of nuclear envelopes about 50 nuclei were isolated, disrupted with blunt glass needles, and centrifuged in LSB at 15,000 *g* for 10 min as described by Jarnik and Aebi (1991).

Direct Conjugation of Antibodies and Wheat Germ Agglutinin with Colloidal Gold

Colloidal gold particles, ~8-nm diam, were prepared by reduction of tetrachloroauric acid with sodium citrate in the presence of tannic acid (Slot and Geuze, 1985). BSA (Boehringer Mannheim GmbH), WGA (Sigma Chemical Co.), and antibodies to NPC proteins (described above) were conjugated to the colloidal gold particles as described by Baschong and Wrigley (1990). After conjugation, the complexes were dialyzed against LSB (see above), and centrifuged at 45,000 *g* for 15 min. The soft pellet was resuspended in LSB and used for labeling isolated nuclei or NE preparations (see below).

Labeling of Isolated Nuclei and NEs

Freshly isolated nuclei were incubated in LSB with WGA or antibodies conjugated directly to colloidal gold (see above) for 20 min to 1 h at room temperature. Next the labeled nuclei were washed in LSB and prepared for electron microscopy as described below. As a control, isolated nuclei were incubated with BSA conjugated to colloidal gold.

Oocytes were also microinjected with colloidal gold-conjugated WGA or antibodies. Accordingly, freshly isolated oocytes were kept in MBS at room temperature, and their cytoplasm was microinjected with 100–200 nl of labeling solution in LSB (see above). After a 1–2 h incubation at room temperature, the nuclei were manually isolated, and their NEs were prepared for electron microscopy as described below.

Labeling of isolated NEs with WGA or antibodies was performed as follows: freshly prepared NE pellets (see above) were incubated for 30 min with colloidal gold–conjugated WGA or antibodies, washed three times with LSB, and Epon embedded for thin sectioning as described below.

Preparation of Specimens for EM

Negative Staining. After labeling, an intact nucleus was transferred with a 5-μl pipette onto a glow-discharged, carbon/collodium-coated EM grid containing a 5-μl drop of LSB. Next the nucleus was disrupted with a pair of blunt glass needles, spread on the EM grid such as to expose its cytoplasmic or nuclear face, washed with LSB, and negatively stained with 0.75% uranyl formate (pH 4.25) for 1 min.

Quick Freezing/Freeze Drying/Rotary Metal Shadowing. A labeled nucleus was deposited, disrupted and spread on a glow-discharged, carbon/colloidon-coated EM grid, and washed with LSB as for negative staining (see above), fixed with 2% glutaraldehyde/1% OsO_4, and quick-frozen as described by Jarnik and Aebi (1991). Frozen specimens were transferred to a BAF-300 freeze-fracture apparatus (Baltec, Fürstentum, Liechtenstein), freeze dried at −80°C for 4 h, rotary shadowed at an elevation angle of 40° with 80 Hz of tantalum/tungsten, and backed with a thin layer of carbon.

Embedding and Thin Sectioning. Labeled nuclei or NE preparations (see above) were Epon embedded as follows: the samples were prefixed with 2% glutaraldehyde/0.2% tannic acid in LSB for 20 min, washed two times with LSB, and postfixed for 1 h with 1% OsO_4 in LSB. Next the chemically fixed samples were dehydrated and embedded in Epon 812 resin as described by Jarnik and Aebi (1991). Thin sections were cut on a Reichert Ultracut ultramicrotome (Reichert-Jung Optische Werke, Vienna, Austria) using a diamond knife. The sections were collected on carbon/collodium-coated EM grids, stained with 6% uranyl acetate for 15 min, and poststained with 2% lead citrate for 1 min (Milloning, 1961).

Electron Microscopy

Specimens were examined in a Hitachi H-8000 transmission electron microscope (Hitachi Ltd., Tokyo, Japan) operated at an acceleration voltage of 100 kV (for negatively stained and metal shadowed specimens) or 75 kV (for embedded/thin-sectioned specimens). Micrographs were recorded on Kodak SO-163 film. Stereo pairs were recorded by tilting the goniometer stage by ±10°. Magnification calibration was performed according to Wrigley (1968) using negatively stained catalase crystals.

Results

Antibodies against NPC Proteins

We have investigated the mutual interactions and location of a group of NPC proteins detected with a monoclonal antibody, QE5. This antibody was derived from spleen cells of a mouse immunized with a high-salt detergent extract of rat liver NEs. Such extracts have previously been shown to be enriched in NPC proteins (Snow et al., 1987). By immunofluorescence, this antibody labeled the NE of all eukaryotic cells so far tested, including human, rat, mouse, dog, chicken, *Xenopus*, *Saccharomyces*, *Schizosaccharomyces*, and *Leishmania*. The punctate labeling patterns observed were reminiscent of those previously seen with antibodies directed against members of the O-linked NPC glycoprotein family (Snow et al., 1987).

As documented in Fig. 1 *a*, immunoprecipitation analyses performed with the monoclonal QE5 antibody on TX extracts obtained from ^{35}S-labeled BHK cells revealed different polypeptides depending on the washing condition used. After washes in high-stringency SDS/TX buffer (i.e., 0.1% SDS, 0.5% Triton X-100, 100 mM NaCl), a single polypeptide with an apparent molecular weight of 170 kD was present in the immunoprecipitates (Fig. 1 *a*, lane *1*). Sequence analysis of a human cDNA encoding this protein (EMBL accession number Z25535; McMorrow et al., 1994) indicated that it corresponds to NUP153, a rat O-linked NPC glycoprotein recently described by Sukegawa and Blobel (1993). However, when the QE5 immunoprecipitates were washed in low-stringency TX buffer (i.e., 0.5% Triton X-100, 500 mM NaCl), five additional polypeptides with apparent molecular weights of 250, 75, 62, 58, and 54 kD were revealed (Fig. 1 *a*, lane *3*). Like NUP153, these polypeptides were only solubilized by nonionic detergents in the presence of high salt (i.e., 500 mM NaCl; Fig. 2 *a*, lane *1*), indicating that they too represent NPC proteins. As illustrated in Fig. 1 *b*, a similar set of proteins was detected when QE5 immunoprecipitates of TX extracts obtained from either rat liver NEs (Fig. 1 *b*, lane *2*) or *Xenopus laevis* A6 (*X.l.*A6) cells (Fig. 1 *b*, lane *6*) were washed in low-stringency TX buffer. However, the *X.l.*A6 polypeptide corresponding to p250 runs closer to NUP153.

The appearance of the additional polypeptides in the QE5 immunoprecipitates that have been washed in low-stringency TX buffer can be accounted for by either cross-reactivity or co-precipitation in a complex. In the latter case, such a complex might reflect mutual interactions of these proteins within the intact NPC. To address this question, QE5 was used to immunoblot QE5 immunoprecipitates. As documented in Fig. 1 *b*, when QE5 immunoprecipitates of TX extracts obtained from either rat liver NEs or *X.l.*A6 cells that had been washed in low-stringency TX buffer were probed with QE5, the only bands labeled above background corresponded to NUP153, plus the 250- and 62-kD polypeptides (Fig. 1 *b*, lanes *1* and *5*). In either case the 75-, 58-, and 54-kD bands were not labeled. The three polypeptides recognized by QE5 (i.e., p250, NUP153, and p62) contain terminal *N*-acetylglucosamine moieties, since: (*a*) they can be enzymatically galactosylated with galactosyltransferase and UDP-[^{3}H]galactose (Fig. 1 *c*); and (*b*) they bind WGA (data not shown)—both diagnostic hallmarks of the members of the O-linked NPC glycoprotein family originally described by Halt et al. (1987) and Snow et al. (1987). The 75-, 58-, and 54-kD polypeptides are apparently not recognized by QE5, nor do they appear to possess terminal *N*-acetylglucosamine residues detectable in the galactosylation assay (see Fig. 1 *c*, lane *1*). Taken together, these findings suggest that the presence of the 75-, 58-, and 54-kD polypeptides in the QE5 immunoprecipitates indicates association with one or more of NUP153, p250, or p62 (see below).

Three additional antibodies have been produced for biochemical and immunolocalization studies: (*a*) a polyclonal rabbit antibody raised against a synthetic peptide representing residues 921 to 930 of human NUP153 (McMorrow et al., 1994) being far removed from the QE5 epitope which has been mapped to a region lying between residues 1,301

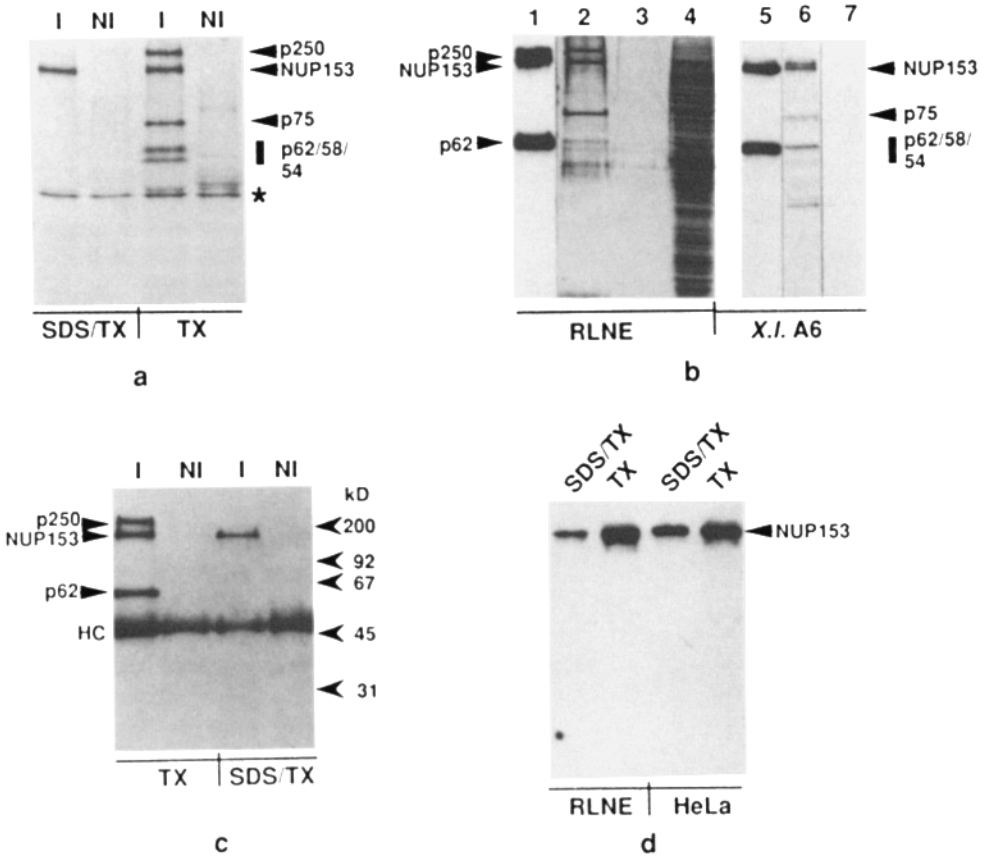


Figure 1. The monoclonal QE5 antibody recognizes three O-linked NPC glycoproteins, p250, NUP153, and p62. (*a*) Immunoprecipitation analysis of ^{35}S-labeled BHK cells with QE5. QE5 immunoprecipitates (*I*) of TX extracts obtained from ^{35}S-labeled BHK cells were subjected to high-stringency washes in SDS/TX buffer or low-stringency washes with TX buffer (see Materials and Methods). Samples were analyzed by SDS-PAGE followed by fluorography. Control nonimmune (*NI*) samples were processed in parallel. Immunoprecipitates washed in high-stringency SDS/TX buffer contained only NUP153 whereas those washed in low-stringency TX buffer contained in addition p250, p75, p62, p58, and p54. p62 and p58 are not well resolved on this gel. (*) A non specific band found in both immune and nonimmune lanes. (*b*) Western blot analysis of rat liver nuclear envelope (*RLNE*) and *Xenopus laevis* A6 cell (*X.l.A6*) QE5-immunoprecipitates probed with QE5. RLNEs were extracted in TX buffer (lane *4*; revealed by silver staining), immunoprecipitated with QE5, and the resulting immunoprecipitate was washed in low-stringency TX buffer. Samples were analyzed by SDS-PAGE and silver stained (lane *2*) or immunoblotted with QE5 (lane *1*). A silver stained nonimmune control precipitate is shown in lane *3*. 50× the amount used in lane *4* was used for lanes *1–3*. QE5 immunoprecipitated p250, NUP153, p75, p62, p58, and p54 (lane *2*), but only p250, NUP153, and p62 were detected by QE5 on the Western blot (lane *1*). TX extracts obtained from ^{35}S-labeled *X.l.* A6 cells were immunoprecipitated with QE5, and the resulting immunoprecipitate was washed in low-stringency TX buffer. Samples were analyzed by SDS-PAGE followed by fluorography (lane *6*) or immunoblotted with QE5 (lane *5*). A fluorograph of a nonimmune control precipitate is shown in lane *7*. The QE5-immunoprecipitate and Western blot of *X.l.* A6 cell extracts (lanes *6* and *5*) exhibit a very similar spectrum of proteins to the mammalian samples (lanes *1* and *2*). However the *X.l.* A6 cell polypeptide corresponding to p250 runs closer to NUP153 (lanes *5* and *6*). (*c*) In vitro galactosylation of QE5 immunoprecipitates from unlabeled BHK cells using UDP-[^{3}H]-galactose and galactosyl transferase. The immunoprecipitates were subjected to either high-stringency (SDS/TX buffer) or low-stringency (TX buffer) washes prior to galactosylation. Samples were fractionated by SDS-PAGE and detected by fluorography. Only p250, NUP153, and p62 are labeled by this procedure, indicating that they are glycoproteins containing GlcNac. The immunoglobulin heavy chain (*HC*, which is present in a large molar excess) was labeled too since it possesses N-linked oligosaccharides which exhibit terminal GlcNac residues at a low frequency (Savvidou et al., 1984). (*d*) Western blot analysis of RLNE and HeLa cell QE5 immunoprecipitates probed with an anti-peptide antibody against NUP153. QE5 immunoprecipitates (using a cross-linked protein G affinity matrix) of TX extracts obtained from RLNEs and HeLa cells were washed using either high-stringency SDS/TX or low-stringency TX buffers. Samples were fractionated by SDS-PAGE, transferred to nitrocellulose, and probed with an anti-peptide antibody against NUP153. Only NUP153 was detected by this antibody in the Western blots of both high-stringency SDS/TX and low-stringency TX QE5 immunoprecipitates, confirming that QE5 recognizes NUP153.

and 1,319 (McMorrow, I., and B. Burke, unpublished observations); (*b*) a polyclonal guinea pig antibody raised against affinity-isolated and gel-purified rat liver NUP153; and (*c*) a polyclonal guinea pig antibody raised against gel-purified rat liver p250. By immunofluorescence, these three antibodies exhibit distinct NE labeling similar to that seen with QE5 (Fig. 3) and other NPC antibodies (Snow et al., 1987). As documented in Fig. 1 *d*, the anti-NUP153 anti-peptide antibody labeled a single 170-kD band on Western blots of QE5 immunoprecipitates obtained from rat liver NEs or HeLa cells that had been washed in either high-stringency SDS/TX or low-stringency TX buffer. These data confirm that the 170-kD polypeptide recognized by the monoclonal QE5 antibody is indeed NUP153. As illustrated in Fig. 2 *c*, immunoprecipitation analyses performed with the polyclonal guinea pig anti-NUP153 antibody after both high-stringency SDS/TX and low-stringency TX washes revealed only NUP-153 (Fig. 2 *c*, lanes *1* and *2*). While the polyclonal guinea pig anti-p250 antibody precipitated only p250 after high-stringency SDS/TX washes (Fig. 2 *c*, lane *3*), it also precipitated p75 under low-stringency TX wash conditions (Fig. 2 *c*, lane *4*). However, this antibody labeled only p250 in Western blots (Fig. 2 *c*, lane *5*), suggesting that p250 and p75 may be associated in a complex (see below). As documented in Fig. 2 *c* (lane *5*), this antibody labeled a single band of 250 kD on Western blots of QE5 immunoprecipitates, indi-

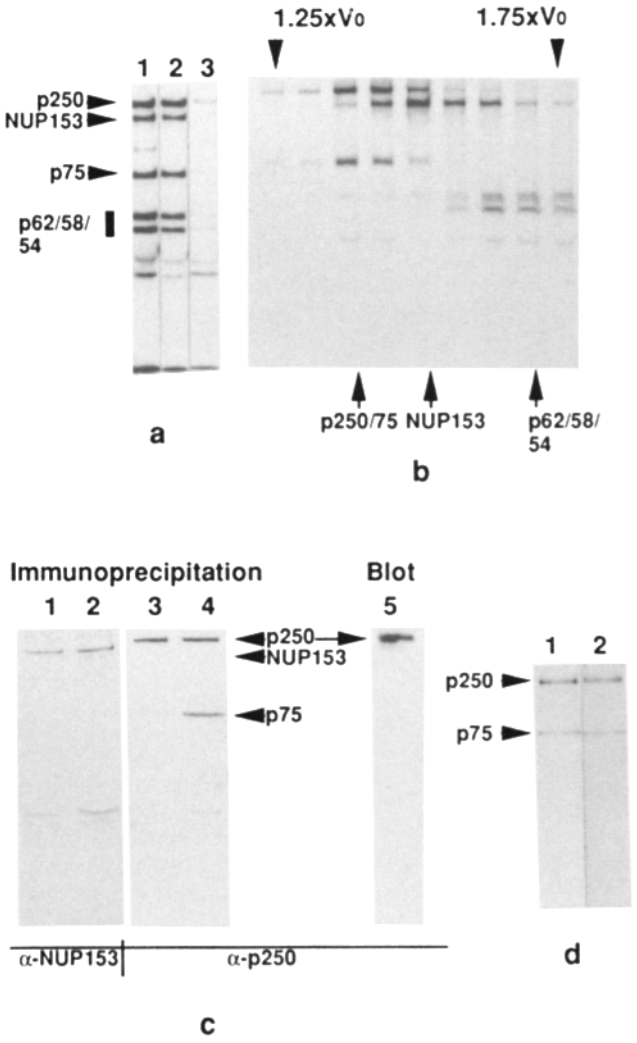


Figure 2. Identification of three discrete complexes containing the NPC proteins recognized by the monoclonal QE5 antibody. (*a*) Immunoprecipitation analysis of ^{35}S-labeled BHK cells with QE5. After labeling with ^{35}S, cells were either extracted with TX buffer (which contains 500 mM NaCl; see Materials and Methods) to yield a TX extract (lane *1*), or with a low-salt extraction buffer (TX buffer containing 100 mM NaCl instead of 500 mM NaCl). The low-salt extract was separated by centrifugation into a low-salt supernatant (lane *3*) and a low-salt pellet (containing nuclei) which was subsequently re-extracted in TX buffer (lane *2*). Each sample was immunoprecipitated with QE5, and the resulting immunoprecipitates were washed in low-stringency TX buffer. Samples were analyzed by SDS-PAGE followed by fluorography. All proteins immunoprecipitated by QE5 are solubilized with high-salt conditions (lanes *1* and *2*) indicating that they are NPC proteins. (*b*) A TX extract of ^{35}S-labeled BHK cells (i.e., lane *1* in *a*) was fractionated on a Sephacryl S-400 column using TX buffer. Each fraction was immunoprecipitated with QE5 followed by low-stringency TX washes, and analyzed by SDS-PAGE followed by fluorography. Fractions eluted between 1.25× and 1.75× the void volume (V_o) of the column are shown. Three complexes (indicated by *upward pointing arrows*) are distinguished: p250/p75, NUP153 and p62/p58/p54. (*c*) Immunoprecipitation analysis of ^{35}S-labeled NRK cells with polyclonal antibodies against either gel purified NUP153 (lanes *1* and *2*) or gel purified p250 (lanes *3* and *4*). Immunoprecipitates were washed with high-stringency (SDS/TX, lanes *1* and *3*) or low-stringency (TX, lanes *2* and *4*) buffer, and analyzed by SDS-PAGE followed by fluorography. The anti-NUP153 antibody precipitated only NUP153 under both high- and low-stringency conditions (lanes *1* and *2*). p75 was found in the anti-p250 immunoprecipitates only under low-stringency wash conditions (lane *4*). The low molecular weight bands are nonspecific bands found in both immune and nonimmune immunoprecipitates (not shown). A Western blot of a QE5-immunoprecipitate (which had been washed in low-stringency TX buffer, as in lane *3* of Fig. 1 *a*) probed with the anti-p250 antibody (lane *5*) revealed labeling of only p250 and not p75. (*d*) Anti-p250 immunoprecipitates of TX extracts obtained from ^{35}S-labeled NRK cells either before (lane *1*) or after (lane *2*) fractionation on a Sephacryl S-400 column. Each immunoprecipitate was washed in low-stringency TX buffer, and analyzed by SDS-PAGE followed by fluorography. Lane *2* corresponds to a pool of the peak p250 fractions in *b*. Both, p250 and p75 were presented in low-stringency immunoprecipitates before and after fractionation on the Sephacryl S-400 column.

cating that both the anti-p250 and the QE5 antibodies recognize the same 250-kD protein.

NUP153, p250, and p62 Are Constituents of Three Distinct Complexes

Since p75, p58 and p54 do apparently not contain epitopes recognized by the monoclonal QE5 antibody, their appearance in immunoprecipitates suggests that they are associated with p250, NUP153, or p62. To resolve this issue, TX extracts from metabolically labeled BHK cells were fractionated on a Sephacryl S-400 column, and each fraction was subjected to immunoprecipitation analysis with the QE5 antibody employing low-stringency TX wash conditions (Fig. 2 *b*). Accordingly, three peaks of immunoprecipitable proteins were observed: (*a*) NUP153 was found to elute as a homo-oligomer with a Stokes radius of ~9.7 nm and a molecular mass of ~1 MD. (*b*) p250 and p75 co-eluted in a single peak closer to the void volume indicating that these two proteins were contained in a complex with a molecular mass of at least 1.5–2.0 MD. (*c*) p62, p58, and p54 were found in the third peak suggesting that these three proteins were also associated in a distinct complex with a Stokes radius of ~6.5 nm and a molecular mass of ~500 kD. This third complex is identical in polypeptide composition and size to the WGA-binding p62 complex previously identified and characterized by Finlay et al. (1991) which has been shown to be essential for nuclear protein import.

To obtain further evidence that p250 and p75 are indeed associated, we used the polyclonal anti-p250 antibody for immunoprecipitation analysis of TX extracts obtained from ^{35}S-labeled NRK cells. Either before (Fig. 2 *d*, lane *1*) or after (Fig. 2 *d*, lane *2*) fractionation on a Sephacryl S-400 column, both p250 and p75 were present in immunoprecipitates following low-stringency TX washes. However, as documented in Fig. 2 *c* (lane *3*), when high-stringency SDS/TX wash conditions were employed, p75 was released and only p250 was recovered in the immunoprecipitates. Under identical high-stringency SDS/TX wash conditions, the polyclonal antibody raised against gel-purified rat liver NUP153 immunoprecipitated only NUP153 (Fig. 2 *c*, lane *1*). Taken together, these findings are consistent with co-immunoprecipitation of p250 and p75 due to association within a distinct complex.

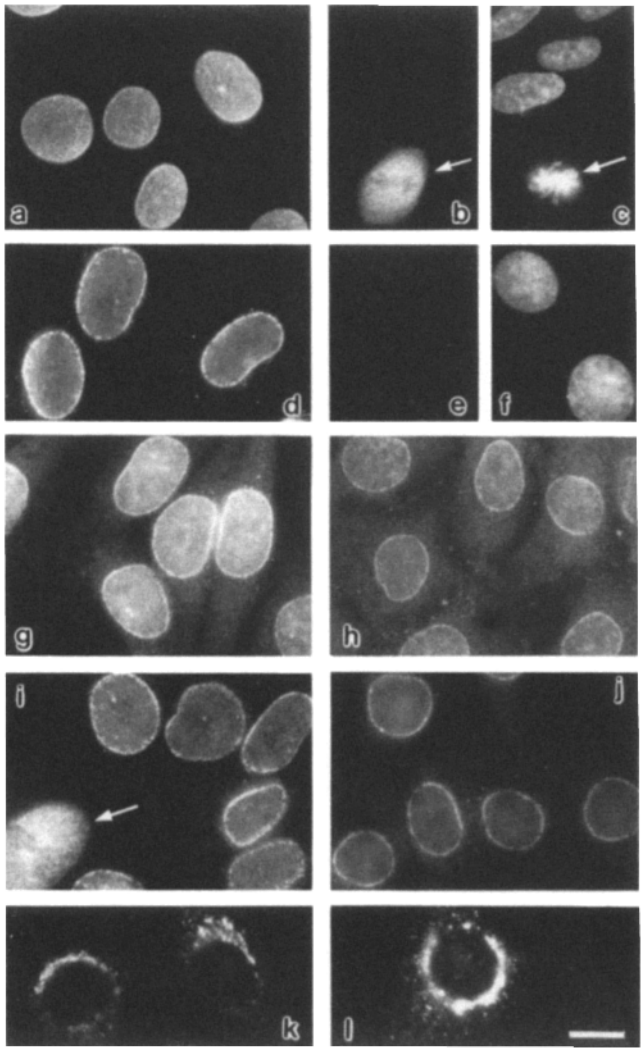


Figure 3. Indirect immunofluorescence micrographs of fixed NRK cells permeabilized either with 0.2% Triton X-100 (left hand column, *a*, *d*, *g*, *i*, and *k*) at room temperature, or with 0.004% digitonin on ice (right hand column, *b*, *c*, *e*, *f*, *h*, *j*, and *l*). Cells were labeled with an anti-peptide antibody against lamin A (*a* and *b*), an anti-peptide antibody against NUP153 (*d* and *e*), a polyclonal antibody against p250 (*g* and *h*), the monoclonal QE5 antibody (*i* and *j*), and AD7, a monoclonal antibody against a cytoplasmically disposed Golgi protein (*k* and *l*). (*c* and *f*) show the same fields as *b* and *e* stained with Hoechst dye to reveal nuclei and chromosomes. The arrow indicates a metaphase cell permeabilized with digitonin which is labeled by the anti-lamin antibody; interphase cells, in which the nuclear envelope is intact, are unlabeled. Bar, 10 μm.

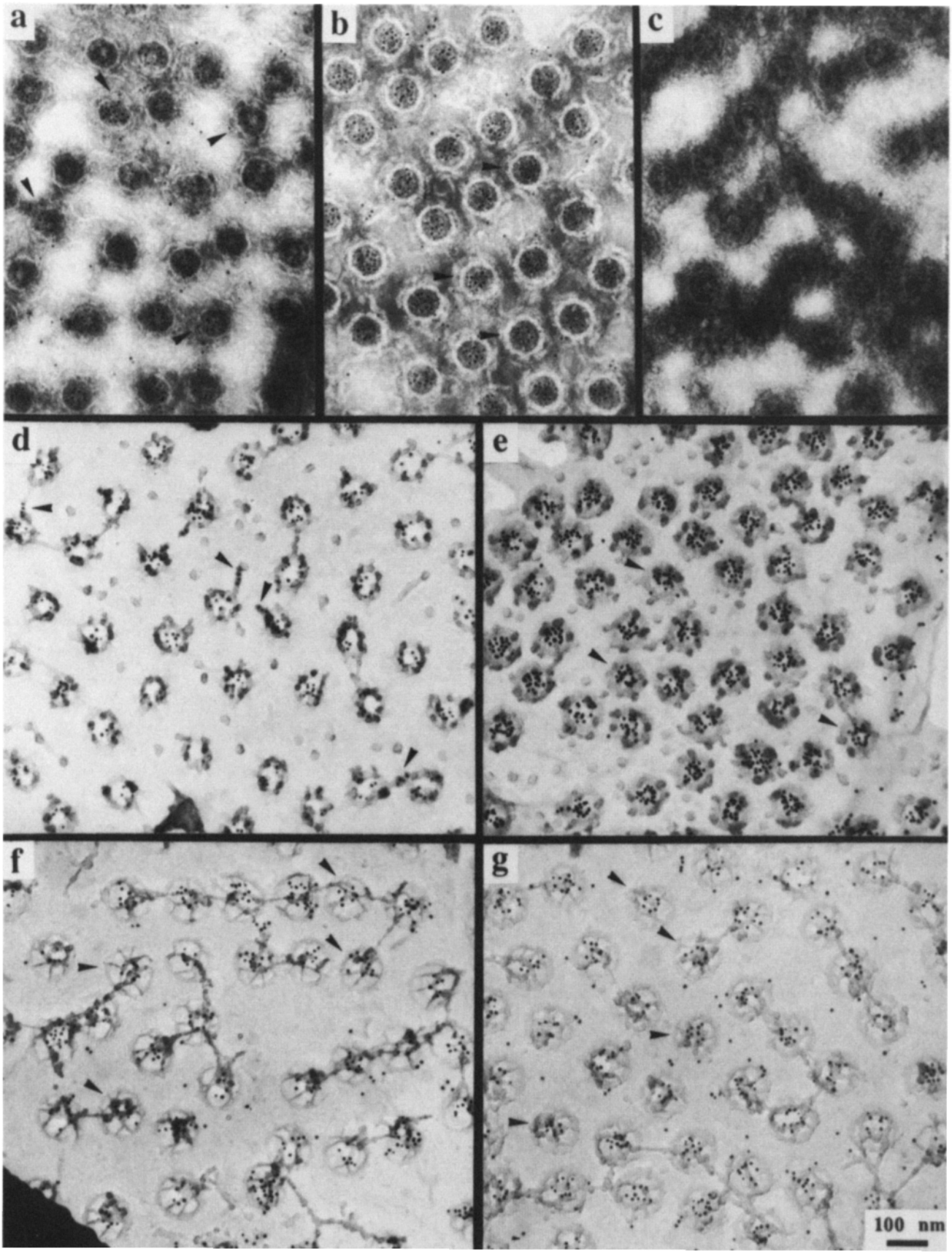
NUP153 Is Located At the Nuclear Face, and p250 at the Cytoplasmic Face of the NPC

To determine on which side of the NE distinct NPC proteins were located, we have combined immunofluorescence microscopy with differential permeabilization of NRK cells. For this purpose, fixed NRK cells were permeabilized either with 2% Triton X-100 at room temperature, or with 0.004% digitonin on ice. The latter conditions have been shown to only permeabilize the plasma membrane while leaving the NE intact (Adam et al., 1990). Therefore, in digitonin-permeabilized cells antibodies do not have access to the nuclear interior, whereas all compartments are accessible to antibodies following Triton X-100 permeabilization. This differential permeabilization was confirmed using antibodies against nuclear lamin A, and p200 (a cytoplasmically disposed protein of the Golgi apparatus). Accordingly, as illustrated in Fig. 3 (*a* and *b*) labeling of the nuclear lamina was only observed with Triton X-100–permeabilized cells but was absent with digitonin-permeabilized cells. In contrast, as shown in Fig. 3 (*k* and *l*), the Golgi-specific antibody labeled cells treated with either of the detergents indicating that the plasma membrane was rendered permeable to antibodies by both detergents. When similar experiments were performed using the anti-peptide antibody against NUP153 (Fig. 3, *d* and *e*) and the anti-p250 antibody (Fig. 3, *g* and *h*), it was found that NUP153 was labeled only in Triton X-100–permeabilized cells (Fig. 3 *d*), whereas p250 could be labeled in cells permeabilized with either detergent (Fig. 3, *g* and *h*). QE5, not surprising since it recognized p250 in addition to NUP153, was found to label cells permeabilized with both detergents (Fig. 3, *i* and *j*). Taken together, these findings confirm previous reports that NUP153 is located at the nucleoplasmic face of the NPC (Sukegawa and Blobel, 1993; Cordes et al., 1993), whereas p250, and by implication p75 since it is in a complex with p250, must at least be located at the cytoplasmic face of the NPC.

The Monoclonal Antibody QE5 Labels Both the Cytoplasmic Filaments and the Terminal Ring of the Nuclear Baskets

To locate distinct NPC proteins within the 3-D structure of the NPC, we have used the monoclonal QE5 antibody in conjunction with several EM preparation methods. For this purpose, isolated *Xenopus* nuclei were incubated with QE5 conjugated to 8-nm-diam colloidal gold. The labeled nuclei were spread on an EM grid and either negatively stained or quick-frozen/freeze-dried/rotary metal-shadowed as described in Materials and Methods. For comparison, nuclei were also incubated with gold-conjugated WGA or BSA. As illustrated in Fig. 4 *a*, the monoclonal QE5 antibody which recognizes three NPC polypeptides in Western blots (p250, NUP153, and p62; see above and Fig. 1 *b*), yielded a distinct labeling pattern of the NPCs in negatively stained preparations. Gold particles were concentrated at about two locations: (*a*) the center of the pore; and (*b*) the periphery of the NPC. WGA which specifically binds to the members of the O-linked NPC glycoprotein family (Snow et al., 1987; Holt et al., 1987), revealed similar labeling of negatively stained NPCs (Fig. 4 *b*). In contrast, no significant labeling of the NPCs was observed when nuclei were incubated under the same conditions with gold-conjugated BSA (Fig. 4 *c*).

To more specifically localize the QE5 epitopes to distinct NPC components, the same labeling experiments were combined with quick freezing/freeze drying/rotary metal shadowing, an EM preparation method that can distinguish between the cytoplasmic and the nuclear face of the NE and preserves both the cytoplasmic filaments and the nuclear baskets (Jarnik and Aebi, 1991). In agreement with the immunofluorescence data (see above and Fig. 3), the gold-conjugated QE5 antibody specifically labeled both the cytoplasmic (Fig. 4 *d*) and the nuclear (Fig. 4 *f*) face of the NPC.


a
b
c
d
e
f
g
100 nm

Although the amounts of gold label appear to be approximately the same on both the cytoplasmic (Fig. 4 *d*) and the nuclear (Fig. 4 *f*) metal replica, when visualized in stereo (Fig. 5) most of the gold particles were actually located at the cytoplasmic side (see Fig. 5 *c*) which due to their strong contrast were also seen within nuclear metal replicas. The relatively small amount of label present at the nuclear side is due to the fact that intact isolated nuclei were incubated with gold-conjugated QE5 thereby preventing these probes to have access to nuclear epitopes. When the experiments were done by either treating the nuclei with Triton X-100 prior to incubation with the antibody, or on the EM grid after spreading the NEs, the extent of labeling at the nuclear face of the NPC was significantly increased. However, Triton-treated nuclei yielded poorly preserved NPCs by quick freezing/freeze drying/rotary metal shadowing (see Jarnik and Aebi, 1991), whereas labeling of spread NEs on the grid yielded a very high background. The sparse labeling observed on the nuclear side of quick-frozen/freeze-dried/rotary metal-shadowed NEs after labeling intact nuclei probably occurred after opening and spreading the NEs on the EM grid, or if some NPCs lost their central channel complex during incubation and/or preparation thereby allowing the antibody–gold complexes to enter the nucleus through unplugged pores. Consistent with this explanation, when labeled intact nuclei were Epon embedded and thin sectioned, the nuclear side of the NEs exhibited very sparse labeling. For embedding/thin sectioning, the extent of labeling of the nuclear side could be significantly improved by either incubating Triton X-100–treated nuclei, or by labeling bulk preparations of isolated NEs.

As illustrated in Fig. 5 *a*, on the cytoplasmic face of the NPC, gold particles were concentrated (*a*) at the cytoplasmic filaments, and (*b*) down in the pore disposed towards the nucleoplasm (see also Fig. 4 *d*, and selected examples in Fig. 6). As can best be appreciated in stereo pairs, frequently the labeled filaments bend either to the side and adhere to filaments of adjacent NPCs (see Fig. 5 *a*, *large arrowhead*), or they bend inward so as to reach down into the pore (see Fig. 5 *a*, *small arrowheads*). On the nuclear face of the NPC, the gold particles were found associated with the nuclear baskets (see Figs. 4 *f*, 5 *b*, and selected examples in Fig. 6). As documented in Fig. 5 *b*, the exact location of the label was at the terminal ring of the nuclear baskets. Although in projection images (i.e., Fig. 4 *f* or when Fig. 5 *b* is inspected without stereo) gold particles seem to be associated with other components of the baskets, or in some cases they appear to just be "hanging in the air" rather than being associated with a distinct mass density of the nuclear baskets, when looked at in stereo, these labels are clearly located at the cytoplasmic face of the NPC (see Fig. 5 *c*). Similarly, the more centrally located gold particles observed at the cytoplasmic face which appear to reside down in the pore (see Figs. 4 *d* and 5 *a*) are predominantly bound to the terminal ring of the nuclear baskets which have been pushed into the pore upon adsorption to the specimen support.

At first glance, WGA–gold revealed a labeling pattern similar to that of QE5–gold in negatively stained preparations (see Fig. 4, *a* and *b*). This was not a surprising finding in light of the fact that QE5 recognizes three distinct members of the O-linked NPC glycoprotein family, namely p250, NUP153, and p62. As revealed by quick freezing/freeze drying/rotary metal shadowing (Figs. 4 *g* and 6 *b*), gold-conjugated WGA also labeled the terminal ring of the nuclear baskets. However at the cytoplasmic face, the labeling pattern with WGA differed somewhat from that seen with QE5 in that no significant labeling was found at the cytoplasmic filaments with WGA–gold (see Fig. 4 *e* and selected examples in Fig. 6). At variance with QE5, the labeling with WGA–gold was concentrated in or at the center of the pore disposed towards the cytoplasm. This difference could also be detected by careful inspection of negatively stained preparations: with QE5, in addition to the more central labeling of the NPC which was very much the same as with WGA, a significant amount of peripheral labeling was depicted (Fig. 4 *a*, *arrowheads*) which was almost absent with WGA (Fig. 4 *b*, *arrowheads*).

NUP153 Is a Constituent of the Nuclear Baskets, and p250 Is a Constituent of the Cytoplasmic Filaments of the NPC

From the QE5-labeling pattern it is possible to infer the location of NUP153 and p250 to distinct peripheral NPC components. Since from the immunofluorescence data (see above and Fig. 3) we can infer that NUP153 is located at the nuclear face of the NPC, and since the only NPC component significantly labeled by QE5 on this side of the NPC is the terminal ring of the basket, it follows that NUP153 must at least be a constituent of this distinct peripheral NPC component. To confirm this conclusion, we performed EM labeling studies with the anti-peptide antibody specific for NUP153. As documented in Figs. 6 *f* and 7 *b*, this antibody labeled exclusively the terminal ring of the nuclear baskets in both quick-frozen/freeze-dried/rotary metal-shadowed (Fig. 6 *f*) and Epon-embedded/thin-sectioned (Fig. 7 *b*) NE prepara-

Figure 4. Comparison of *Xenopus laevis* oocyte nuclear envelopes labeled with the monoclonal QE5 antibody (*a*, *d*, and *f*), WGA (*b*, *e*, and *g*) and BSA (*c*). Intact *Xenopus* oocyte nuclei were incubated with QE5, WGA, or BSA conjugated to 8-nm-diam colloidal gold, spread on an EM grid and prepared for EM by negative staining (*a–c*) or quick freezing/freeze drying/rotary metal shadowing (*d–g*) as described in Materials and Methods. Micrographs of both cytoplasmic (*d* and *e*) and nuclear (*f* and *g*) NE faces were recorded when quick freezing/freeze drying/rotary metal shadowing was used. The monoclonal QE5 antibody that recognizes p250, NUP153 and p62, specifically labeled the NPCs as is shown by negative staining (*a*). WGA that binds to members of the O-linked NPC glycoprotein family also labeled the NPCs (*b*), while no significant labeling of the NPCs was observed with BSA–gold (*c*). The NPCs were more peripherally labeled with QE5 (*a*, *arrowheads*) than with WGA. Quick freezing/freeze-drying/rotary metal shadowing revealed that QE5 labeled the cytoplasmic filaments (*d*, *arrowheads*; see also stereo pair in Fig. 5 *a* and selected examples in Fig. 6 *c*), and the terminal ring of the nuclear baskets (*f*, *arrowheads*; see also stereo pair in Fig. 5 *b* and selected examples in Fig. 6 *d*). WGA–gold also labeled the terminal ring of the nuclear baskets (*g*, *arrowheads*; see also selected examples in Fig. 6 *b*), whereas it labeled the center and the inner annulus of the NPC at the cytoplasmic face (*b* and *e*, *arrowheads*; see also selected examples in Fig. 6 *a*). Bar, 100 nm.

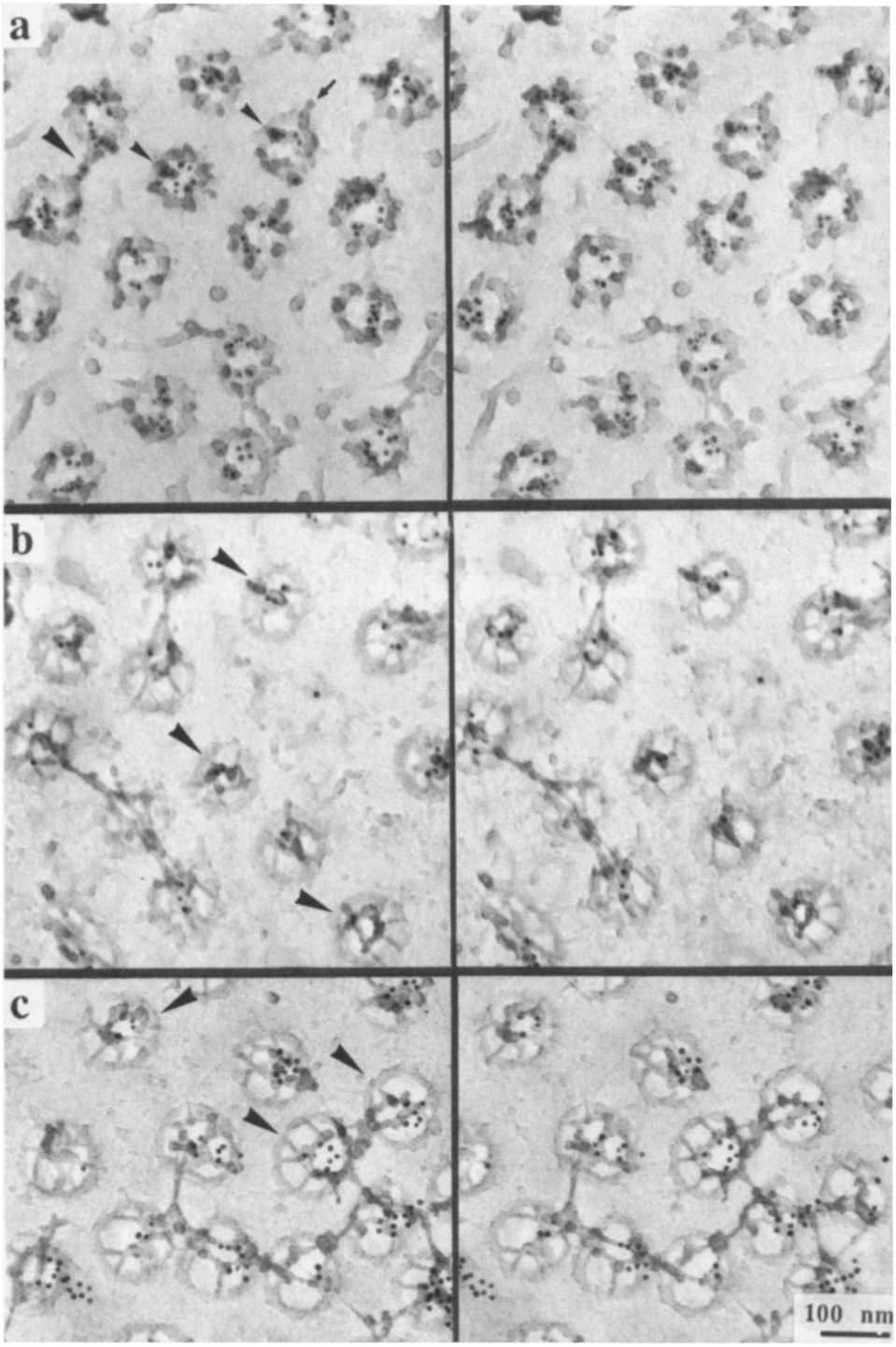


Figure 5. Stereo pairs of the cytoplasmic (*a*) and nuclear (*b* and *c*) faces of *Xenopus* oocyte NEs labeled with the monoclonal QE5 antibody. Intact *Xenopus* oocyte nuclei were incubated with QE5 conjugated to 8-nm-diam colloidal gold, spread on an EM grid and quick-frozen/freeze-dried/rotary metal-shadowed as described in Materials and Methods. The epitopes for the monoclonal QE5 antibody at the cytoplasmic face are located predominantly at the cytoplasmic filaments, whereas at the nuclear face they are located at the terminal ring of the nuclear baskets. The large arrowhead in *a* points to a labeled cytoplasmic filament that has bent to the side and adhered to a filament of an adjacent NPC, whereas the small arrowheads mark filaments that have bent inward to reach down into the pore. Notice that in one NPC one cytoplasmic filament is bending to the side (*small arrow*), whereas a second filament is bending inward to reach down into the pore. The arrowheads in *b* point to labeled nuclear baskets, and in *c* to nuclear baskets that in projection appear to be labeled but when looked at in stereo the gold particles are actually at the cytoplasmic side. Bar, 100 nm (a-c).

tions. Similarly, since p250 is exposed on or accessible from the cytoplasmic face of the NPC (see above and Fig. 3), it is conceivable that it represents a constituent of the cytoplasmic filaments or is located somewhere between the central channel complex and the cytoplasmic ring. As documented in Figs. 6 *e* and 7 *a*, based on immunogold labeling using the polyclonal anti-p250 antibody, p250 is definitely a constituent of the cytoplasmic filaments.

Discussion

Considerable progress has been made in the past few years in the elucidation of the 3-D structure of the NPC. Several independent 3-D reconstructions of the NPC have now been computed (Unwin and Milligan, 1982; Hinshaw et al., 1992; Akey and Radermacher, 1993), and a consensus model of its basic framework is beginning to emerge (reviewed by Panté and Aebi, 1993, 1994). In addition, distinct peripheral NPC components such as the cytoplasmic and the nuclear ring, the cytoplasmic filaments and the nuclear baskets have been visualized by a number of different EM specimen preparation and imaging methods (Ris, 1991; Jarnik and Aebi, 1991; Goldberg and Allen, 1992; Ris and Malecki, 1993; Goldie et al., 1994).

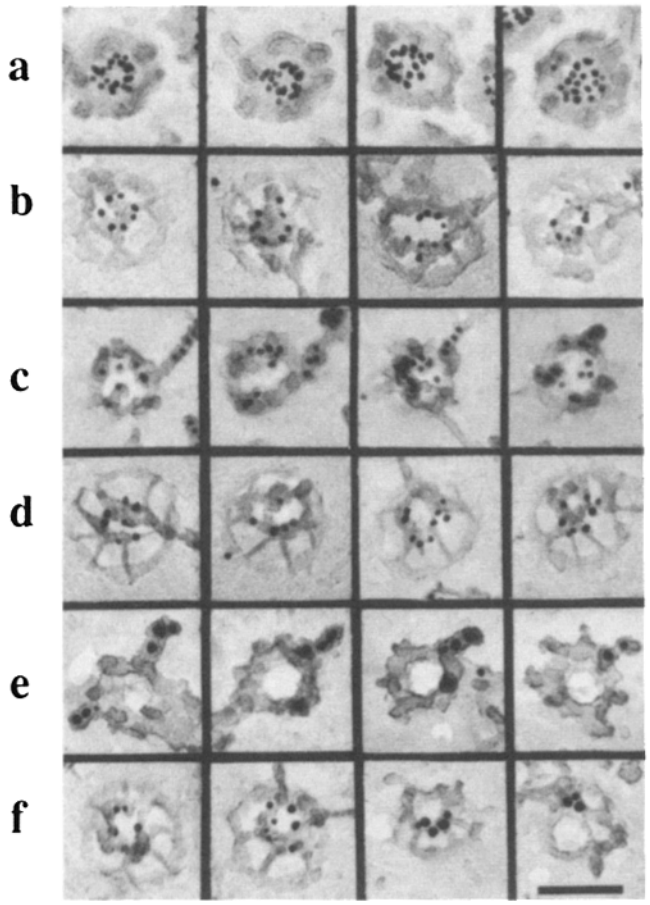


Figure 6. Gallery of selected examples of NPCs labeled with WGA (*a* and *b*), the monoclonal QE5 antibody (*c* and *d*), a polyclonal antibody against p250 (*e*), and an anti-peptide antibody against NUP153 (*f*). Intact *Xenopus* oocyte nuclei were incubated with gold-conjugated WGA or antibodies, spread on an EM grid and quick-frozen/freeze-dried/rotary metal-shadowed as described in Materials and Methods. WGA labeled the center and the inner annulus of the NPC at the cytoplasmic face (*a*), and the terminal ring of the nuclear baskets at the nuclear face (*b*). The monoclonal QE5 antibody which recognizes both p250 and NUP153 labeled the cytoplasmic filaments (*c*) and the terminal ring of the nuclear baskets (*d*). Whereas the anti-p250 antibody only labeled the cytoplasmic filaments (*e*), the anti-NUP153 anti-peptide antibody only labeled the terminal ring of the nuclear baskets (*f*). Bar, 100 nm.

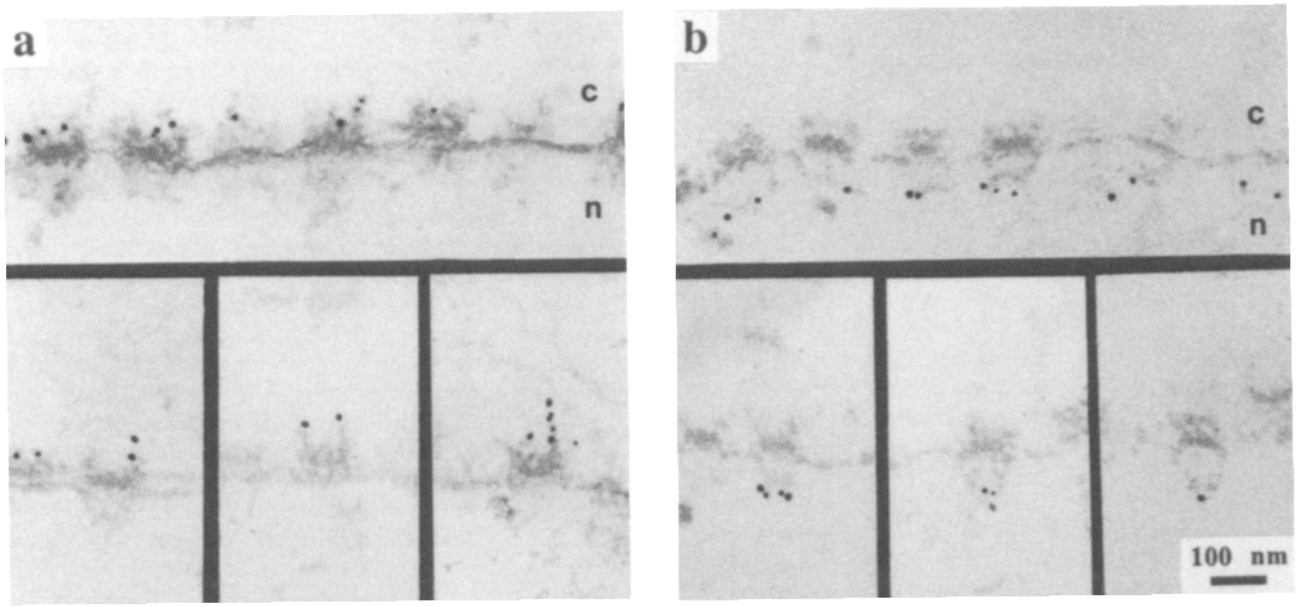


Figure 7. Localization of p250 and NUP153 by embedding and thin sectioning. Cross-sections and selected examples of Triton X-100 treated *Xenopus* oocyte nuclei labeled with anti-p250 antibody (*a*), and anti-NUP153 anti-peptide antibody (*b*). Nuclei were incubated with the antibodies conjugated to 8-nm colloidal gold prior to embedding and thin sectioning (see Materials and Methods). In agreement with the quick freezing/freeze drying/rotary metal shadowing results in Fig. 6, the cross-sections revealed that the anti-p250 antibody labeled the cytoplasmic filaments, whereas the anti-NUP153 anti-peptide antibody labeled the terminal ring of the nuclear baskets. (*c*, cytoplasmic; *n*, nuclear side of the nuclear envelope). Bar, 100 nm.

Despite of this progress, the "molecular" architecture of the NPC has remained elusive. Based on its mass of about 120 MD, with the mass of the asymmetric unit (i.e., one half-spoke) being on the order of that of a ribosome (i.e., ~3.3 MD), it is estimated that the NPC may be built of multiple copies (i.e., 8 or 16, because of the 822 symmetry of the basic framework) of 100 or more different polypeptides. In addition, it is conceivable that very much like the ribosome, the NPC also harbors specific RNAs. To date, roughly one dozen NPC proteins from diverse organisms have been cloned and sequenced, and perhaps 20–30 putative NPC proteins have been identified, isolated or characterized. However, correlation of these proteins with distinct structural components of the NPC has been largely unsuccessful. One reason for this slow progress has been the fact that the few immunolocalization studies which have so far been carried out, have relied upon conventional embedding/thin sectioning preparations in which distinct NPC components are either not well resolved or poorly preserved.

Notwithstanding this, two NPC proteins have recently been localized to distinct, peripheral NPC components: (*a*) NUP153, a member of the O-linked NPC glycoprotein family was initially localized exclusively to the nuclear side of the NE (Snow et al., 1987; Sukegawa and Blobel, 1993), and more recently to intranuclear filaments attached to the nuclear periphery of the NPC (Cordes et al., 1993). (*b*) NUP180, a non-WGA binding NPC protein has been localized to the cytoplasmic ring and possibly the cytoplasmic filaments (Wilken et al., 1993). Based on a number of biochemical data, gp210 must be associated with the nuclear membrane embracing the NPC (Gerace et al., 1982; Wozniak et al., 1989; Greber et al., 1990). Hence it has been suggested that: (*a*) this NPC protein may be a constituent of the lumenal domain of the spokes (Hinshaw et al., 1992; Akey and Radermacher, 1993); and (*b*) it may be a constituent of the distinct "knobs" that have been visualized in thin sections and which extend radially from the spokes into the perinuclear space (Jarnik and Aebi, 1991). Direct labeling studies, however, have yet to confirm this putative localization.

In the present investigation we have employed a variety of colloidal gold–conjugated probes to localize NPC glycoproteins in quick-frozen/freeze-dried/rotary metal-shadowed *Xenopus* oocyte NE preparations which reveal the peripheral structures of the NPC, including the cytoplasmic filaments and the nuclear baskets (Jarnik and Aebi, 1991). By this preparation procedure we have been able to demonstrate that NUP153 is a component of the terminal ring of the nuclear baskets, and p250, also a member of the O-linked NPC glycoprotein family, is a constituent of the cytoplasmic filaments. Although these distinct peripheral NPC components have previously been identified using various specimen preparation methods (Jarnik and Aebi, 1991; Ris, 1991; Goldberg and Allen, 1992; Goldie et al., 1993), the issue of whether these represent authentic NPC components has never been completely resolved. The present immunolocalization studies now provide strong evidence that the cytoplasmic filaments and the nuclear baskets must represent structural components of the NPC, since biochemical and immunofluorescence analyses have clearly indicated that both NUP153 and p250 are strongly and specifically associated with the nuclear envelope (see Figs. 2 and 3).

As is the case with many antibodies raised against NPC components (Davis and Blobel, 1986; Snow et al., 1987; Sukegawa and Blobel, 1993), the monoclonal QE5 antibody used in this study was polyspecific in that it recognized three distinct NPC polypeptides, p250, NUP153, and p62. However, this cross-reactivity did not involve the GlcNac moieties present on these proteins, since QE5 recognized bacterially expressed NUP153 (which is not O-glycosylated; data not shown), and its binding to NUP153 was unaffected by in vitro galactosylation. Instead, mapping studies suggest that the QE5 epitope is composed, at least in part, of one of the XFXFG pentapeptide repeats (McMorrow, I., and B. Burke, unpublished observations), hence the recognition of three distinct NPC proteins by QE5. The use of this antibody in immunoprecipitation studies and Western blot analyses of extracts of both whole cells and purified NEs has enabled us to establish the mildest conditions under which p250, NUP153, and p62 may be released from the NPCs. Under such conditions, NUP153 is recovered as a homo-oligomer, whereas p250 and p62 are recovered in two separate complexes in association with additional NPC proteins. Our findings with p62 confirm previous work by Finlay et al. (1991) who identified a 550–600-kD p62 complex containing at least two additional polypeptides of 58 and 54 kD which was required for nuclear protein import. The 75-kD protein found in association with p250 appears to represent a novel NPC protein. It is not a member of the O-linked NPC glycoprotein family since it cannot be galactosylated in vitro to any significant extent and since it is not recognized by the broad-specificity anti-NPC glycoprotein antibody RL1 (Snow et al., 1987). Thus far, limited amino acid sequence analysis has revealed no obvious homology to related proteins (data not shown). This approach of using available NPC antibodies to immunoaffinity-purify complexes released from the NPC under relatively mild conditions may provide a more general tool of identifying new NPC polypeptides while at the same time lending insight into their mutual interactions.

Our observation that NUP153 is a constituent of the terminal ring of the nuclear baskets is in general agreement with a recent report by Cordes et al. (1993) that this protein is a constituent of intranuclear filaments attached to the nuclear periphery of the NPC. However, this earlier study also found NUP153-immunoreactive material in long nuclear filament bundles attached to the NPC. Using two independent antibodies (the monoclonal QE5 antibody and the anti-NUP153 anti-peptide antibody) we have found no evidence for such a localization. It is conceivable that these filament bundles may represent nuclear baskets which have been sheared during sample preparation, or perhaps cross-reacting nuclear matrix elements sharing a common epitope with NUP153.

We have previously reported that the nuclear baskets lose their structural integrity upon chelation of divalent cations, and stabilization/restoration of the baskets is most effective in the presence of zinc (Jarnik and Aebi, 1991). Since NUP153 is unique among the characterized NPC proteins in that it contains four zinc fingers and is clearly located at the nuclear baskets, it suggests a potential involvement in the opening/closing of the baskets. In addition, Sukegawa and Blobel (1993) who have also immunolocalized NUP153 to the nuclear face of the NPC, have provided evidence that recombinant NUP153 zinc fingers are capable of binding DNA in a zinc-dependent manner.

Our immunolocalization of p250 to the cytoplasmic filaments is consistent with our immunofluorescence micros-

copy data of selectively permeabilized cells (see Fig. 3), which indicate that this protein is exposed on the cytoplasmic face of the NPC. At the present time we have no amino acid sequence data for p250 and therefore cannot draw any firm conclusions concerning its conformation. However, given its molecular weight of about 250 kD, a single copy would be large enough to extend from the base of a cytoplasmic filament to its tip. For instance, human erythrocyte spectrin heterodimers which are built from two antiparallel, extended subunits only slightly smaller than p250, exhibit a contour length of approximately 100 nm (Shotton et al., 1979), about the length of the cytoplasmic filaments. Our biochemical analysis has demonstrated that p75 resides in a complex with p250, implying that p75 too must be a constituent of the cytoplasmic filaments of the NPC, although the exact location of this protein remains uncertain. It is conceivable that p75 is a constituent of the filament base thereby being involved in their attachment to the cytoplasmic ring or a distinct spoke domain.

At least three other NPC proteins have recently been localized to the cytoplasmic face of the NPC: (*a*) the nonglycosylated NUP180 (Wilken et al., 1993); (*b*) the 210-kD O-linked glycoprotein NUP214 (Kraemer et al., 1994), which was first identified by Snow et al. (1987) and reported to be a homologue of the human CAN protein (Kraemer et al., 1994), a putative oncogene product of 214 kD associated with myeloid leukemogenesis (Von Lindern et al., 1992); and (*c*) p260 (Byrd, D., N. Panté, U. Aebi and L. Gerace, unpublished results), a nonglycosylated NPC protein which is a homologue of human tpr (translocated promoter region; Gerace, L., personal communication), a 260-kD protein whose gene has been implicated in the activation of the *met* and *raf* proto-oncogenes (Mitchell and Cooper, 1992). Of these three NPC proteins, only NUP180 has thus far been identified to a distinct NPC component, i.e., the cytoplasmic ring and possibly the cytoplasmic filaments (Wilken et al., 1993). Since NUP180 is very prone to proteolysis, it is conceivable that this protein is actually a proteolytic fragment derived from a 260-kD polypeptide recognized by the anti-NUP180 autoimmune antibody used by Wilken et al. (1993), indicating that this 260-kD protein may actually represent p260/tpr. The O-linked glycoprotein p250 recognized by our monoclonal QE5 antibody that we have identified as a constituent of the cytoplasmic filaments is definitely distinct from p260/tpr which is: (*a*) not glycosylated; and (*b*) not recognized by the monoclonal QE5 antibody (Gerace, L., personal communication). On the other hand, p250 may correspond to the O-linked glycoprotein NUP214/CAN, however, this identification will require sequence analysis or the use of specific anti-peptide antibodies. A possible interaction between p250 and p260/tpr remains to be determined.

Since the monoclonal QE5 antibody used in this study recognized three members of the O-linked NPC glycoprotein family (p250, NUP153, and p62), we were interested to determine whether colloidal gold-conjugated WGA yielded a similar or perhaps more extensive labeling pattern of NPCs. As QE5, WGA-gold labeled the terminal ring of the nuclear baskets where NUP153 resides (see above and Figs. 4 to 7). However, distinct from QE5 labeling no significant WGA labeling was found at the cytoplasmic filaments. This difference may at first seem paradoxical since we have shown that the O-linked glycoprotein p250 is a constituent of the cytoplasmic filaments. However, it is conceivable that glycosylation within these filaments may be relatively low compared to other regions of the NPC. It is also possible that GlcNac residues within these filaments may not be easily accessible to WGA-gold.

When viewed in projection (i.e., in negatively stained preparations; see Fig. 4 *b*), the NPCs labeled with WGA-gold revealed gold particles in or at the pore at two distinct locations: (*a*) at low radii between 3.6 and 12.7 nm; and (*b*) at higher radii between 18.2 and 36.2 nm (Fig. 8; and Panté et al., 1992). Although some of the label observed in such projection images could be due to WGA-gold being bound to the terminal ring of nuclear baskets which may have been pushed into the pore upon adsorption to the EM grid and/or negative staining (Jarnik and Aebi, 1991), based on embedded/thin sectioned and quick-frozen/freeze-dried/rotary metal-shadowed preparations (data not shown) most of these labels actually reside in or at the pore, and are disposed towards the cytoplasmic side of the NPC. This bimodal distribution for the binding sites of WGA was previously reported by Akey and Goldfarb (1989) in projection images of frozen hydrated NPCs. However, these authors reported that both types of labeling occurred as "arcs" or "semicircles," and that the labeling at higher radii was significantly less frequent than that at lower radii. In contrast with their findings, we did not observe an arc- or semicircle-like labeling pattern at lower radii, and in our preparations both types of labeling occurred with a similar frequency (see Figs. 4 *b* and 8) even when using the same short incubation times reported by Akey and Goldfarb (1989). It is conceivable that these differences may be due to the different sample preparation methods used, i.e., negatively stained versus frozen hydrated NEs.

In summary, we have localized two O-linked NPC glycoproteins to distinct peripheral NPC components, namely the cytoplasmic filaments in the case of p250, and the terminal ring of the nuclear basket in the case of NUP153. Furthermore, we have shown that additional glycoproteins, possibly

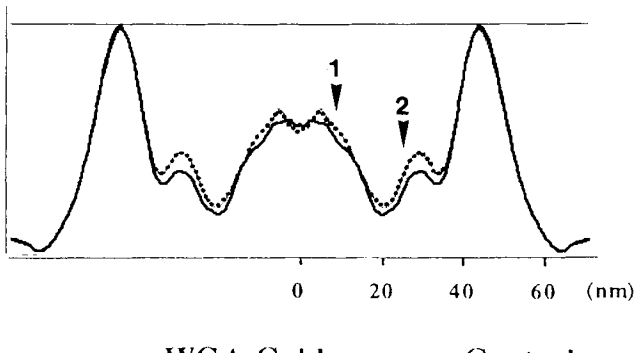


Figure 8. Radial density profiles of correlation averaged WGA-gold labeled (——) and unlabeled control (- - - -) NPCs as shown in Fig. 4 *b*. Two distinct WGA binding regions can be depicted: one about the central channel complex at radii between 3.7 and 12.7 nm, and a second at the inner annulus of the NPC at radii between 18.2 and 36.2 nm. Notice that high density values in this plot represent stain excluded areas (i.e., protein), whereas low density values represent metal (i.e., negative stain and/or gold label). Therefore the two WGA binding regions have densities lower than those of the corresponding unlabeled control NPCs. This figure was kindly provided by Dr. M. Jarnik (National Institutes of Health, Bethesda, MD).

including p62, are present in the region of the inner annulus of the NPC. While the specific functional role of individual NPC glycoproteins remains unknown, it is nevertheless clear that they must somehow be involved in nucleocytoplasmic transport. The role of NUP153 and p250 in this process remains a matter of speculation. However, our findings that p250 (in association with p75) is a constituent of the cytoplasmic filaments places this polypeptide in a pivotal location to interact with incoming macromolecules, e.g., during their docking to the NPC. Thus the p250/p75 complex might form the initial docking site for nuclear localization signal bearing proteins upon binding to specific cytoplasmic nuclear localization signal receptors. This hypothesis can now be tested by employing specific antibodies directed against p250.

We would like to thank Dr. M. Jarnik for providing Fig. 8. We are grateful to Ms. U. Sauder for her important help with embedding and thin sectioning. Ms. H. Frefel and Ms. M. Zoller are thanked for their expert photographic work.

This work was supported by the M.E. Müller Foundation of Switzerland, by grants from the Swiss National Science Foundation and the Human Frontier Science Program Organization to U. Aebi, and National Institutes of Health grant No. GM38556 to B. Burke, and by the Department of Education of the Kanton Basel-Stadt. R. Bastos is the recipient of a fellowship from the Ministerio de Educacion y Ciencia (Spain).

Received for publication 14 March 1994 and in revised form 20 April 1994.

References

Adam, S. A., R. Sterne-Marr, and L. Gerace. 1990. Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J. Cell Biol.* 111:807–816.

Akey, C. W. 1989. Interactions and structure of the nuclear pore complex revealed by cryo-electron microscopy. *J. Cell Biol.* 109:955–970.

Akey, C. W., and D. S. Goldfarb. 1989. Protein import through the nuclear pore complex is a multistep process. *J. Cell Biol.* 109:971–982.

Akey, C. W., and M. Radermacher. 1993. Architecture of the *Xenopus* nuclear pore complex revealed by three-dimensional cryo-electron microscopy. *J. Cell Biol.* 122:1–19.

Ash, J. F., D. Louvard, and S. J. Singer. 1977. Antibody-induced linkages of plasma membrane proteins to intracellular actomyosin-containing filaments in cultured fibroblasts. *Proc. Natl. Acad. Sci. USA.* 74:5584–5588.

Baschong, W., and N. G. Wrigley. 1990. Small colloidal gold conjugated to Fab fragments or to immunoglobulin G as high-resolution labels for electron microscopy: a technical overview. *J. Elec. Microsc. Tech.* 14:313–323.

Blobel, G., and V. R. Potter. 1966. Nuclei from rat liver: isolation method that combines purity with high yield. *Science (Wash. DC).* 154:1662–1664.

Burke, B. 1990. On the cell-free association of lamins A and C with metaphase chromosomes. *Exp. Cell Res.* 186:169–176.

Burke, B., G. Griffiths, H. Reggio, D. Louvard, and G. Warren. 1982. A monoclonal antibody against a 135k Golgi membrane protein. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:1621–1628.

Burnette, W. N. 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:195–203.

Carmo-Fonseca, M., H. Kern, and E. C. Hurt. 1991. Human nucleoporin p62 and the essential yeast nuclear pore protein NSP1 show sequence homology and a similar domain organization. *Eur. J. Cell Biol.* 55:17–30.

Coffino, P., and M. D. Scharff. 1971. Rate of somatic mutation in immunoglobulin production by mouse myeloma cells. *Proc. Natl. Acad. Sci. USA.* 68: 219–223.

Cordes, V., I. Waizenegger, and G. Krohne. 1991. Nuclear pore complex glycoprotein p62 of *Xenopus laevis* and mouse: cDNA cloning and identification of its glycosylation region. *Eur. J. Cell Biol.* 55:31–47.

Cordes, V., S. Reidenbach, A. Köhler, N. Stuurman, R. van Driel, and W. W. Franke. 1993. Intranuclear filaments containing a nuclear pore complex protein. *J. Cell Biol.* 123:1333–1344.

Dabauvalle, M.-C., K. Loos, and U. Scheer. 1990. Identification of a soluble precursor complex essential for nuclear pore assembly *in vitro*. *Chromosoma (Berl.).* 100:56–66.

Davis, L. I., and G. Blobel. 1986. Identification and characterization of a nuclear pore complex protein. *Cell.* 45:699–709.

Davis, L. I., and G. Blobel. 1987. The nuclear pore complex contains a family of glycoproteins that includes p62: glycosylation through a previously unidentified cellular pathway. *Proc. Natl. Acad. Sci. USA.* 84:7552–7556.

Davis, L. I., and G. R. Fink. 1990. The NUP1 gene encodes an essential component of the yeast nuclear pore complex. *Cell.* 61:965–978.

Dingwall, C. 1993. Fingers in the pore. *Curr. Biol.* 3:297–299.

Finlay, D. R., E. Meier, P. Bradley, J. Horecka, and D. J. Forbes. 1991. A complex of nuclear pore proteins required for pore function. *J. Cell Biol.* 114:169–183.

Gerace, L. 1992. Molecular trafficking across the nuclear pore complex. *Curr. Opin. Cell Biol.* 4:637–645.

Gerace, L., Y. Ottaviano, and C. Kondor-Koch. 1982. Identification of a major polypeptide of the nuclear pore complex. *J. Cell Biol.* 95:826–837.

Gerace, L., C. Comeau, and M. Benson. 1984. Organization and modulation of nuclear lamina structure. *J. Cell Sci. Suppl.* 1:137–160.

Goldberg, M. W., and T. D. Allen. 1992. High resolution scanning electron microscopy of the nuclear envelope: demonstration of a new, regular, fibrous lattice attached to the baskets of the nucleoplasmic face of the nuclear pores. *J. Cell Biol.* 119:1429–1440.

Goldie, K. N., N. Panté, A. Engel, and U. Aebi. 1994. Exploring native nuclear pore complex structure and conformation by scanning force microscopy in physiological buffers. *J. High Vacc. Tech.* In press.

Greber, U. F., A. Senior, and L. Gerace. 1990. A major glycoprotein of the nuclear pore complex is a membrane-spanning polypeptide with a large lumenal domain and a small cytoplasmic tail. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:1495–1502.

Hallberg, E., R. W. Wozniak, and G. Blobel. 1993. An integral membrane protein of the pore membrane domain of the nuclear envelope contains a nucleoporin-like region. *J. Cell Biol.* 122:513–521.

Harlow, E., and D. Lane. 1988. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. pp. 522–523.

Hinshaw, J. E., B. O. Carragher, and R. A. Milligan. 1992. Architecture and design of the nuclear pore complex. *Cell.* 69:1133–1141.

Holt, G. D., and G. W. Hart. 1986. The subcellular distribution of terminal N-acetylglucosamine moieties. *J. Biol. Chem.* 261:8049–8057.

Holt, G. D, C. M. Snow, A. Senior, R. S. Haltiwanger, L. Gerace, and G. W. Hart. 1987. Nuclear pore complex glycoproteins contain cytoplasmically disposed O-linked N-acetylglucosamine. *J. Cell Biol.* 104:1157–1164.

Hurt, E. C. 1990. Targeting of a cytosolic protein to the nuclear periphery. *J. Cell Biol.* 111:2829–2837.

Hurt, E. C, A. McDowall, and T. Schimmang. 1988. Nucleolar and nuclear envelope proteins of the yeast *Saccharomyces cerevisiae*. *Eur. J. Cell Biol.* 46:554–563.

Jarnik, M., and U. Aebi. 1991. Towards a more complete 3-D structure of the nuclear pore complex. *J. Struct. Biol.* 107:291–308.

Kreis, T. E. 1986. Microinjection of antibodies against the cytoplasmic domain of vesicular stomatitis virus glycoprotein block in transport to the cell surface. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:931–941.

Kraemer, D., R. W. Wozniak, G. Blobel, and Radu, A. 1994. The human CAN protein, a putative oncogene product associated with myeloid leukemogenesis, is a nuclear pore complex protein that faces the cytoplasm. *Proc. Natl. Acad. Sci. USA.* 91:1519–1523.

Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

McMorrow, I. M., R. Bastos, H. Horton, and B. Burke. 1994. Sequence analysis of a cDNA encoding a human nuclear pore complex protein, hnup153. *Biochim. Biophys. Acta.* 1217:219–223.

Milloning, G. 1961. A modified procedure for lead staining of thin sections. *J. Biophys. Biochem. Cytol.* 11:736–739.

Mitchell, P. J., and C. S. Cooper. 1992. Nucleotide sequence analysis of human *tpr* cDNA clones. *Oncogene.* 7:383–388.

Panté, N., and U. Aebi. 1993. The nuclear pore complex. *J. Cell Biol.* 122: 977–984.

Panté, N., and U. Aebi. 1994. Towards understanding the 3-D structure of the nuclear pore complex at the molecular level. *Curr. Opin. Struct. Biol.* 4: 187–196.

Panté, N., M. Jarnik, and U. Aebi. 1992. Towards a 3-D molecular model of the nuclear pore complex. *Proc. EUREM.* 92:321–324.

Radu, A., G. Blobel, and R. W. Wozniak. 1993. Nup155 is a novel nuclear pore complex protein that contains neither repetitive sequence motifs nor reacts with WGA. *J. Cell Biol.* 121:1–9.

Reichelt, R., A. Holzenburg, E. L. Buhle, M. Jarnik, A. Engel, and U. Aebi. 1990. Correlation between structure and mass distribution of the nuclear pore complex, and of distinct pore complex components. *J. Cell Biol.* 110:883–894.

Ris, H. 1991. The 3-D structure of the nuclear pore complex as seen by high voltage electron microscopy and high resolution low voltage scanning electron microscopy. *EMSA Bull.* 21:54–56.

Ris, H., and M. Malecki. 1993. High-resolution field emission scanning electron microscope imaging of internal cell structures after Epon extraction from sections: a new approach to correlative ultrastructural and immunocytochemical studies. *J. Struct. Biol.* 111:148–157.

Savvidou, G., M. Klein, A. A. Grey, K. J. Dorrington, and J. P. Carver. 1984. Possible role for peptide-oligosaccharide interactions in differential oligosaccharide processing at asparagine-107 of the light chain and asparagine-297 of the heavy chain in a monoclonal IgG1k. *Biochemistry.* 23:3736–3740.

Shotton, D. M., B. E. Burke, and D. Branton. 1979. The molecular structure of human erythrocyte spectrin: biophysical and electron microscopic studies. *J. Mol. Biol.* 131:303–329.

Slot, J. W., and H. J. Geuze. 1985. A new method of preparing gold probes for multiple-labeling cytochemistry. *Eur. J. Cell Biol.* 38:87–93.

Snow, C. M., A. Senior, and L. Gerace. 1987. Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. *J. Cell Biol.* 104:1143–1156.

Starr, C. M., M. D'Onofrio, M. K. Park, and J. A. Hanover. 1990. Primary sequence and heterologous expression of nuclear pore glycoprotein p62. *J. Cell Biol.* 110:1861–1871.

Sukegawa, J., and G. Blobel. 1993. A nuclear pore complex protein that contains zinc finger motifs, binds DNA, and faces the nucleoplasm. *Cell.* 72:29–38.

Taggart, R. T., and I. M. Samloff. 1982. Stable antibody-producing murine hybridomas. *Science (Wash. DC).* 219:1228–1230.

Unwin, P. N. T., and R. A. Milligan. 1982. A large particle associated with the perimeter of the nucleare pore complex. *J. Cell Biol.* 93:63–75.

Von Lindern, M., M. Fornerod, S. van Baal, M. Jaegle, T. de Wit, A. Buijs and G. Groveld. 1992. The Translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, *dek* and *can*, and the expresion of a chimeric, leukemia-specific *dek-can* mRNA. *Mol. Cell Biol.* 12:1687–1697.

Wilken, N., U. Kossner, J.-L. Senécal, U. Scheer, and M.-C. Dabauvalle. 1993. Nup180, a novel nuclear pore complex protein localizing to the cytoplasmic ring and associated fibrils. *J. Cell Biol.* 123:1345–1354.

Wimmer, C., V. Doye, P. Grandi, U. Nehrbass, and E. C. Hurt. 1992. A new subclass of nucleoporins that functionally interact with nuclear pore protein NSP1. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:5051–5061.

Wozniak, R. W., E. Bartnik, and G. Blobel. 1989. Primary structure analysis of an integral membrane glycoprotein of the nuclear pore. *J. Cell Biol.* 108:2083–2092.

Wrigley, N. G. 1968. The lattice spacing of crystalline catalase as an internal standard of length in electron microscopy. *J. Ultrastruct. Res.* 24:454–464.