Nuclear Pore Complex Structure Analyzed by Immunogold EM with Human Autoantibodies (Invited)

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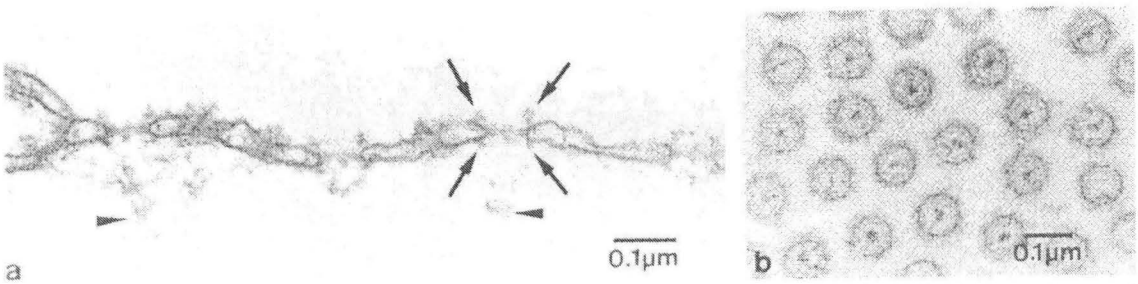
1. INTRODUCTION

The nuclear envelope is interrupted at numerous sites by nuclear pore complexes (NPC), which provide plasmatic channels through the double-layered nuclear membrane barrier (Figs. 1a,b). To understand the molecular mechanisms involved in the highly selective and energy-requiring bidirectional transport processes through the NPCs, it is essential to understand their architecture and biochemical composition (for recent NPC models see Hinshaw et al., 1992; Akey and Radermacher, 1993). The pore channel is flanked by two coplanar rings or annuli, one attached to the cytoplasmic and one to the nucleoplasmic pore margin (Fig. 1a). Both rings are composed of eight subunits each with octagonal symmetry. Within the pore channel is a central granule or plug (Fig. 1b), believed to be the structural equivalent of the central transporter by which nucleocytoplasmic exchange of macromolecules takes place. Thin protein filaments extend from the NPC into the nuclear interior and cytoplasm, respectively. The nucleoplasmic or inner annulus-attached filaments form long cylindrical arrays or shorter basket-like assemblies (Fig. 1a). In contrast, the filaments extending from the outer annulus into the cytoplasm are much shorter. The functions of these annulus-associated filaments is at present unknown.

Although several protein constituents, collectively termed "nucleoporins", have been described and molecularly characterized, only limited information is presently available on the biochemical nature of a minority of the various gross morphological components of the NPCs (reviewed by Forbes, 1992). A set of 8-10 glycoproteins with molecular masses ranging from 45-210 kD reside centrally within the NPC, and are most likely constituents of the transporter assembly. The members of this glycoprotein family are modified by O-linked N-acetylglucosamine (GlcNAc) residues, and hence bind the plant lectin wheat germ agglutinin (WGA). The most prominent GlcNAc-containing nucleoporin is p62 (Fig. 2), which has been shown to be involved in nuclear RNA export and protein import (for refs. see Forbes, 1992). The proteins of the NPC may be anchored to the pore membrane by integral membrane proteins such as gp210 (Wozniak et al., 1989), or POM121 (Soederqvist and Hallberg, 1994; Fig. 2). Recently a protein (nup153) of the intranuclear annulus-attached filaments has been identified (Fig. 2; Sukegawa and Blobel, 1993; Cordes et al., 1993). Interestingly, nup 153 contains zinc finger motifs; a characteristic of DNA-binding proteins.

Autoantibodies directed against NPC proteins have been found in sera of patients suffering from liver and rheumatic autoimmune diseases (Senécal and Raymond, 1992). Here, we describe the biochemical characterization and localization of two novel NPC proteins using autoimmune antibodies from patients with overlap connective tissue diseases.



a b

Figure 1: Ultrastructure of NPCs as revealed in transverse (a) and tangential (b) sections of nuclear envelopes manually isolated from Xenopus oocytes. The annulus subunits on both sides of the pores are indicated by arrows. Arrowheads denote the inner-annulus attached filaments. The prominent central granules are particularly evident in tangential views (b).

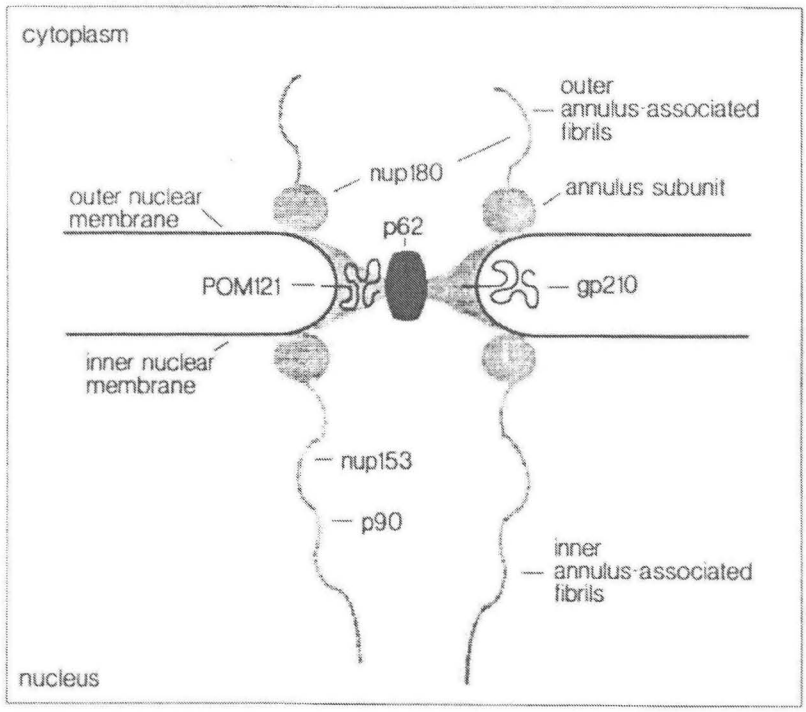


Figure 2: Schematic representation of a NPC, showing the sublocalization of some proteins. For details see text .

2. NUP 180: A NOVEL NUCLEAR PORE COMPLEX PROTEIN LOCALIZING TO THE CYTOPLASMIC ANNULUS AND ASSOCIATED FILAMENTS

Nup180 is an evolutionarily highly conserved nuclear pore complex protein with an estimated molecular mass of 180 kD and an isoelectric point of approximately 6.2 (Wilken al. 1993). It binds neither WGA nor ConA. Based on its extractability from isolated nuclear envelopes with 2 M urea, nup180 is a peripheral membrane protein. Affinity-purified antibodies decorated the NPCs in a striking asymmetrical pattern. Gold particles were almost exclusively associated with the cytoplasmic annulus and the short filaments radiating therefrom into the cytoplasm (Fig. 3a; see also Fig. 2). Microinjection of affinity-purified antibodies into Xenopus oocytes and cultured cells did not affect nuclear protein transport. After addition of the antibodies to Xenopus egg extract, nuclei formed normally around added DNA or chromatin, and were capable of accumulating karyophilic proteins (Wilken et al., 1993). Thus, at the moment we have no positive evidence that nup180 is directly involved in nucleocytoplasmic transport processes. Possibly the NPC-attached cytoplasmic filaments

provide a physical connection between NPCs and the cytoskeleton (Carmo-Fonseca et al., 1987).

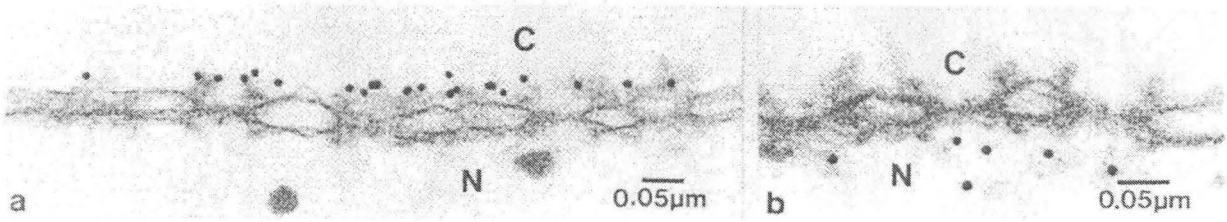


Figure 3: Pre-embedding immunogold EM localization of nup180 and p90. Manually isolated nuclear envelopes from Xenopus oocytes were incubated with affinity-purified antibodies to nup180 (a) or p90 (b) followed by gold-conjugated secondary antibodies. The cytoplasmic annulus and associated filaments are labelled with anti-nup180 (a), whereas antibodies to p90 decorate the tentacle-like nucleoplasmic fibrils (b). N, nucleoplasmic side; C, cytoplasmic side of the nuclear envelope.

3. A PROTEIN OF THE NUCLEOPLASMIC NPC-ATTACHED FILAMENTS

Using the serum from a patient with overlap connective tissue disease, we have found that a mammalian protein of 90 kD (provisionally named p90) with an isoelectric point of 5.5 is a general constituent of the intranuclear NPC-attached filaments. It does not bind WGA. In Xenopus oocytes, the antigen has a slightly lower apparent molecular mass of 80 kD. Immunogold EM localized p90 exclusively to the tentacle like filaments attached to the nucleoplasmic annulus of Xenopus NPCs (Fig. 3b; see also Fig. 2). When "synthetic" nuclei assembled in Xenopus egg extracts were probed with anti-p90, they revealed a distinctly punctate fluorescence of their outer surface indicating that p90 is a component of the newly formed NPCs. We hope that microinjection and immunodepletion experiments of Xenopus egg extract will provide us with some clues as to the function of p90 in NPC assembly or nucleocytoplasmic transport processes.

4. CYTOPLASMIC PORE COMPLEXES: THE ANNULATE LAMELLAE

Pore complexes are present not only in the nuclear envelope, but may also occur in the cytoplasm of numerous cell types in form of annulate lamellae (AL; Fig. 4a; for review see Kessel, 1993). AL are flat membrane cisternae with numerous pore complexes at almost maximal packing density which, morphologically, are indistinguishable from NPCs. AL can be induced in cultured cells by treatment with vinblastine sulfate (Fig. 4b). In contrast to the nucleoporins p62, nup180 and p90, the transmembrane NPC protein gp210 appears to be absent from AL pore complexes. Thus, gp210 is unlikely to serve as a general anchor for the NPC to the pore membrane.

5. BEHAVIOR OF THE PORE COMPLEXES DURING MITOSIS

During mitosis of higher eukaryotic cells, the nuclear envelope, including the lamina and NPCs, goes through a disassembly/assembly cycle. How the "soluble" nuclear envelope components are targeted at telophase to the surface of the chromosomes, and in which order they reassemble into the nascent nuclear envelope, is at present poorly understood. To gain some more insight into the processes involved, we have used a cell free extract of activated Xenopus eggs. This extract is capable of assembling purified DNA or chromatin into nuclear-like structures which share several structural and functional features with authentic interphase nuclei (Fig. 5; for review see Laskey and Leno, 1990). In the presence of limiting amounts of DNA large stacks of AL form spontaneously within 60-90 min (Fig. 5; Dabauvalle et al., 1991). Our results suggest that the extract has the capacity to assemble a certain amount of pore complex-containing membranes either in the form of nuclear envelopes or AL. When chromatin is available for binding the membrane vesicles, nuclear envelopes will form. In a cycling egg extract the AL disintegrate during each mitotic event in step with the nuclear

envelope. We thus have an experimental system at hand which allows us to study the mitotic or soluble forms of NPC components, and the assembly and disassembly pathways of pore complex proteins independent of chromatin.

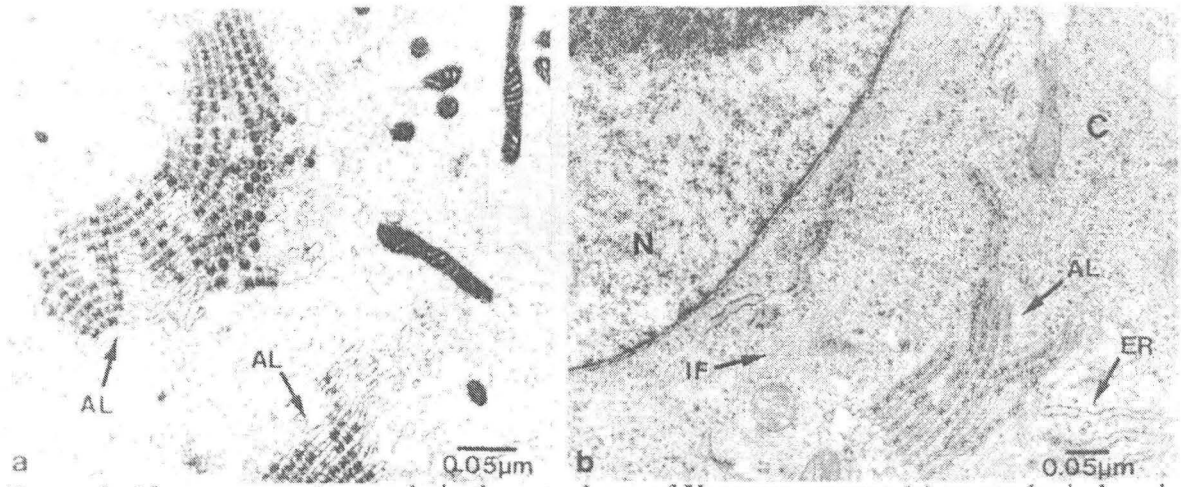


Figure 4: AL occur spontaneously in the cytoplasm of Xenopus oocytes (a) or can be induced in cultured cells by vinblastine sulfate (b; shown is a rat RV cell). N, nucleus; C, cytoplasm; IF, intermediate filaments; ER, endoplasmic reticulum.

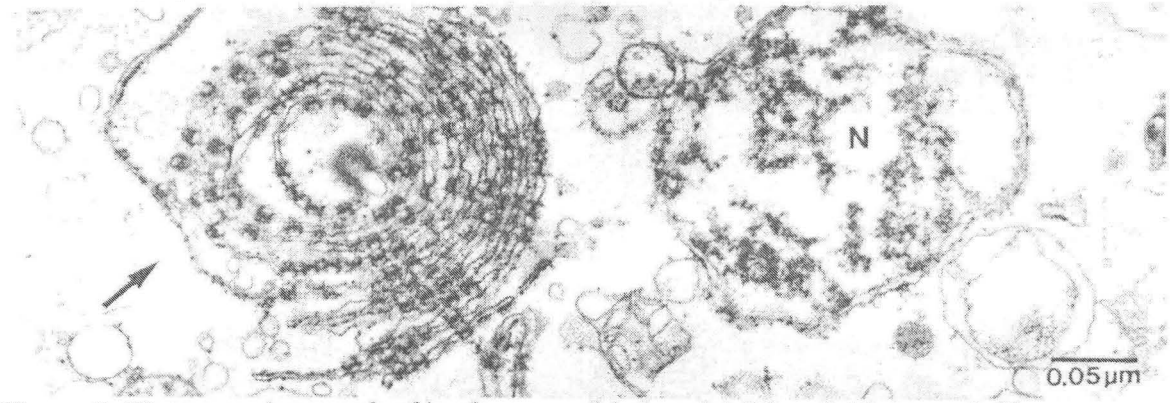


Figure 5: Electron micrograph of in vitro assembled nuclei (N) and AL (arrow). Xenopus egg extract was incubated for 90 min with lambda DNA and processed for EM.

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