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Isolation of Nuclear Pore Complexes in Association with a Lamina

(nuclear envelope/subfractionation/electron microscopy/electrophoresis)

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ABSTRACT **Nuclear pore complexes have been isolated in association with a 150 Å thick lamina by detergent and salt fractionation of nuclear envelopes from rat liver. The pore complexes exhibit characteristic morphology and appear to be attached in a highly specific orientation to the lamina, which extends over relatively large areas. The pore complex-lamina fraction is composed of three major and several minor polypeptides with little or no DNA, RNA, or phospholipid. It is suggested that the association of the pore complexes and the lamina reflects the structural arrangement of the nuclear periphery *in vivo*.**

One of the unique features of eukaryotic cells is the double membrane system surrounding the nucleus in interphase. A striking characteristic of this nuclear envelope is the presence of pores, i.e., circular holes in the double membrane which, for any particular cell type, are of uniform dimension, generally in the range of 400–800 Å in diameter (1, 2). The pores contain material that has been referred to as either annular material or nuclear pore complex (3–7). The ultrastructure of the pore complex has been studied extensively, and several different models have been proposed (8–12). However, little is known of its composition. There are numerous reports that pore complexes are sensitive to proteolysis (13–18) and that they may contain considerable amounts of RNA (19). Finally, their precise function remains unknown. It has been suggested that they are involved in nucleocytoplasmic exchange of macromolecules (3, 4, 20) or in chromatin organization (21, 22).

In this preliminary report we describe the isolation and the partial characterization of a subfraction containing nuclear pore complexes in association with a lamina obtained from isolated rat liver nuclei. It appears that the pore complexes are interconnected and oriented by the lamina. We suggest that this lamina, hitherto not described in hepatic nuclei, corresponds to a similar lamina often observed in nuclei from other sources (23–26).

METHODS

Preparation of a Nuclear Envelope Fraction. Nuclei were prepared from fresh rat liver using a slight modification (27) of the method of Blobel and Potter (28).

Nuclear envelopes were prepared essentially as described by Kay *et al.* (29). Nuclei isolated from approximately 12 rats were incubated at a concentration of 6×10^6 nuclei per ml (approximately 2 mg of protein per ml) at 23° for 15 min in the presence of 8 mM Tris·HCl, pH 8.5, 0.1 mM $MgCl_2$, 11 mM 2-mercaptoethanol, 0.25 M sucrose, and 1 μg/ml of pancreatic DNase I (Boehringer). The reaction was stopped by the addition of an equal volume of cold, double-distilled water, and crude envelopes were collected by centrifugation for 20 min at 20,000 × *g* in a Sorvall angle rotor (SS-34) at 4°. The pellet of crude envelopes was resuspended with 15 ml of 10 mM Tris·HCl, pH 7.5, 0.1 mM $MgCl_2$, 14 mM 2-mercaptoethanol, 0.25 M sucrose to which DNase I was added to a final concentration of 1 μg/ml. After incubation for 20 min at 23°, the reaction was stopped by the addition of 30 ml of cold, double-distilled water. The nuclear envelopes were collected by centrifugation for 20 min at 1000 × *g* in a swinging bucket rotor.

Further Treatment of the Envelope Fraction. (*a*) *Detergent solubilization of the phospholipid.* The nuclear envelopes were suspended in 12 ml of cold 0.25 M sucrose, 50 mM Tris·HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$ to which 3 ml of 10% (v/v) Triton X-100 solution was added. After the mixture was incubated at 0° for 10 min, the suspension was centrifuged for 15 min at 1,000 × *g* at 4°.

(*b*) *$MgCl_2$ solubilization of residual chromatin.* The detergent-treated pellet was resuspended in cold 0.25 M sucrose, 50 mM Tris·HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$ to which sufficient 1 M $MgCl_2$ was then added to yield a final concentration of 0.3 M. The pellet obtained upon centrifugation at 1000 × *g* for 15 min at 4° will be referred to as the pore complex-lamina fraction.

Biochemical Analysis. The composition of each subfraction was analyzed after precipitation with cold trichloroacetic acid, using standard techniques for DNA (30), RNA (31), protein (32), and phospholipid (33, 34). Electrophoretic analysis of reduced and alkylated protein was performed essentially as described by Maizel (35) in the presence of sodium dodecyl sulfate in thin slab gels containing a linear 10–15% gradient of polyacrylamide.

Electron Microscopy. The pore complex-lamina fraction was fixed in suspension in 40 mM triethanolamine·HCl, pH 7.5, 20 mM KCl, 4 mM $MgCl_2$, 2% glutaraldehyde for 1 hr at 0° and postfixed as a pellet for 1 hr at 0° in the same buffer with 0.8% OsO_4 replacing the glutaraldehyde. The pellet was then stained *en bloc* with uranyl acetate in acetate-Veronal buffer (36), dehydrated, and embedded in Epon (37). Thin sections were cut with a diamond knife. The sections were stained with uranyl acetate (38) and lead citrate (39) and viewed in a Siemens Elmiskop 101 at 80 kV.

RESULTS

The primary goal of this work was the isolation of the nuclear pore complex from rat liver nuclei. Nuclear pore complexes have been observed in nuclear membrane fractions isolated by

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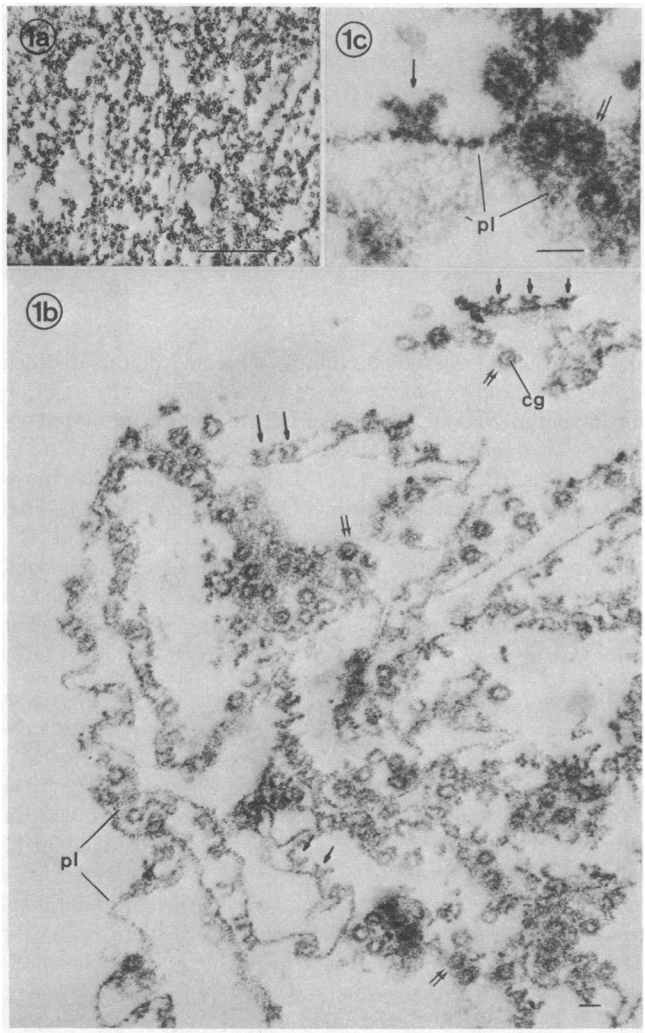

FIG. 1. Electron micrographs of the pore complex-lamina fraction. (a) Low magnification survey. ×10,150. (b) Demonstration of the extent of the lamina and of the arrangement of the pore complexes. ×27,300. (c) Demonstration, in greater detail, of the arrangement of the pore complexes on the lamina. ×63,000. Pore complexes in frontal view, *double arrow;* pore complexes in lateral view, *single arrow;* central granule, *cg;* peripheral lamina, *pl.* The bar denotes 1 μm in (a) and 1000 Å in (b) and (c).

a variety of techniques (29, 40–46). A substantial initial enrichment for pore complexes thus may be achieved by preparing such a fraction. The technique of Kay *et al.* (29), using a repeated digestion with DNase of nuclei at two different alkaline pH values, was chosen because it is rapid and provides a high yield of large nuclear envelopes. The envelopes are essentially nuclear "ghosts," and are readily visible by phase contrast microscopy. Using electron microscopy, we have confirmed (not shown) that envelopes are morphologically well-preserved, containing the ribosome-studded outer nuclear membrane that lies parallel to the inner nuclear membrane with which it is continuous at pores. Pore complexes remain intact in the pores. The inner nuclear membrane has some amorphous material associated with its inner aspect, and there are occasional small aggregates of chromatin present, but the bulk of the nuclear material, i.e., chromatin, nucleoli, etc., is absent.

TABLE 1. *Composition of subfractions during preparation of the pore complex-lamina*

	Protein* (mg)	DNA/ protein	RNA/ protein	PLP†/ protein
Nuclei	150	0.46	0.06	0.035
Nuclear envelope	25	0.30	0.07	0.090
Triton-treated nuclear envelope	16	0.40	0.08	0.006
Pore complex-lamina	2.5	0.04	0.02	—

* Per 99 g wet weight of rat liver.
† PLP = phospholipid.

Previous work indicated that the nuclear pore complex did not require the presence of a membrane for preservation of its integrity, since intact nuclear pore complexes remain attached to membrane-denuded nuclei upon solubilization of the membranes with the nonionic detergent, Triton X-100 (27). Thus, it was of interest to treat the nuclear "ghosts" with Triton X-100 in the hope that the membranes would be solubilized, leaving the pore complexes intact. There was no assurance of this result since it was quite possible that the structural integrity of the pore complex, while not absolutely requiring the presence of both the membrane and the bulk of the chromatin, might require at least one or the other. Thus, Triton X-100 was added to a suspension of nuclear ghosts while the results were monitored in the phase contrast microscope. Surprisingly, the "ghosts" remained even when the concentration of Triton reached 5% (v/v), which should have been more than sufficient to solubilize the membranes (27).

The "ghosts" which thus remained after Triton treatment of the envelope fraction could well have been remnants of the highly condensed peripheral chromatin, which may be relatively DNase-resistant. In attempts to solubilize the chromatin by salt extraction (45), the lowest effective concentration of $MgCl_2$ was used. Large empty sacs of nuclear proportions were still observed in the phase contrast microscope. Many of the sacs were, however, distorted, smaller, and aggregated.

Electron microscopic examination of this fraction (Fig. 1) revealed three readily identifiable structures: annuli, approximately 700–900 Å in diameter (Fig. 1b), reminiscent of nuclear pore complexes in frontal view (especially after detergent treatment of nuclei; see ref. 27); goblet-shaped structures, approximately 650 Å at the stem, reminiscent of pore complexes in lateral view; and an amorphous lamina, approximately 150 Å thick. The contour length of the lamina, which can often be followed for several micrometers (Fig. 1a and b), indicates that it exists as fairly large sheets, perhaps large enough to enclose a nucleus with a single sheet. Occasionally, along such a contour length (Fig. 1b) several goblet-shaped structures, their bases continuous with the lamina, can be seen projecting in the same direction, presumably towards the cytoplasmic side of the lamina. Other views suggestive of pore complexes and lamina in oblique section are common owing to the convolutions of the lamina.

When the plane of sectioning lies parallel to but above the lamina, the annuli are quite distinct (Fig. 1b and c). When the plane of sectioning is actually tangent to the lamina, its granularity is apparent between the annuli (Fig. 1b and c). Occasionally, a dark central granule is observed within the

annuli, further reinforcing the similarity with nuclear pore complexes seen *en face*.

The composition of the material at each stage of the isolation is presented in Table 1. We have experienced some variability with respect to the relative amounts of the constituents owing to the presence of occasional clumps of nuclei that resist complete DNase digestion. However, it can be seen that the Triton solubilized over 95% of the original phospholipid while solubilizing only approximately 30% of the protein. The detergent-solubilized protein presumably consists primarily of membrane and ribosomal proteins. It can also be seen that 90% of the DNA and 75% of the RNA remaining after the detergent treatment are solubilized by the salt treatment. Further efforts to reduce the level of nucleic acids by using higher salt concentrations or subsequent treatment with DNase or RNase have been unsuccessful.

The proteins of each fraction have been analyzed by electrophoresis in the presence of sodium dodecylsulfate in polyacrylamide gradient gels, and the resulting electropherograms are shown in Fig. 2. Slot 4 represents the spectrum of proteins present in whole nuclei. The prominent low-molecular-weight bands (see arrows) have previously been identified as histones by coelectrophoresis with authentic histones.

The nuclear envelopes (slot 3), in contrast, exhibit a marked enrichment for four prominent bands, corresponding to polypeptides with molecular weights of approximately 69,000, 68,000, 66,000, and 50,000, and a somewhat fainter but sharper band migrating only a short distance into the gel. In addition, there are several other polypeptide species present in lesser amounts as well as significant amounts of histones which undoubtedly arise from the chromatin observed in the electron microscope.

Previous work (27) has shown that the 50,000 molecular weight band is a major constituent of the nuclear membrane. Detergent treatment of the nuclear envelopes clearly removes this protein (slot 2) along with the phospholipid. The histones present after detergent treatment undoubtedly remain complexed with the remaining DNA.

Analysis of the pore complex-lamina fraction (slot 1) indicates that the salt treatment extracts most of the histones, as expected from the solubilization of the DNA. The three major bands, of approximately 66,000, 68,000 and 69,000 molecular weight, which remained after detergent solubilization of the membrane, are present, as is the more slowly migrating band of undetermined molecular weight. These bands appear to be present in the same relative amounts as in the original nuclear envelope fraction. The levels of the other bands initially present seem reduced.

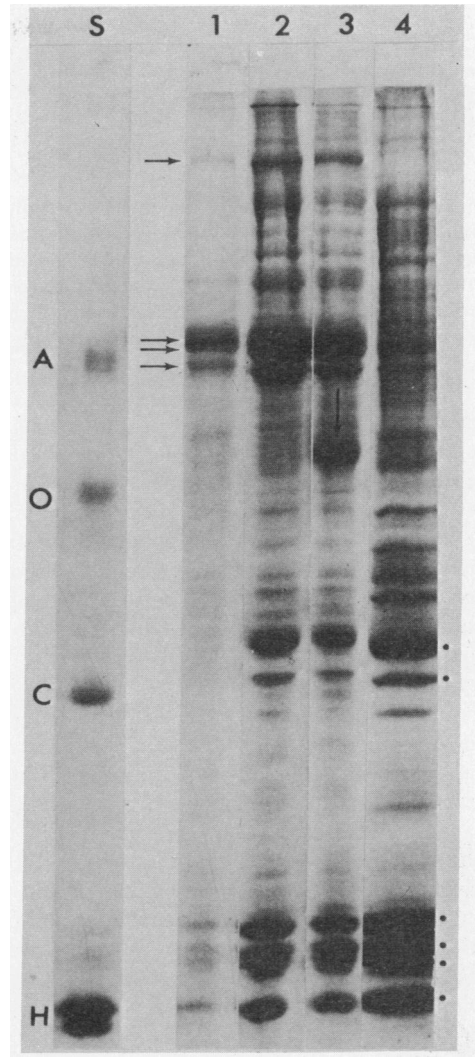

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electropherograms of fractions obtained during preparation of the pore complex-lamina fraction. Slot *1*, pore complex-lamina (horizontal arrows indicate characteristic polypeptides; see *text*); *2*, nuclear envelopes after Triton X-100 treatment; *3*, nuclear envelopes (vertical arrow indicates polypeptide predominant in nuclear membranes and removed by Triton X-100 treatment; see slot *2*); *4*, whole nuclei (dots indicate histones, as determined by coelectrophoresis with authentic histones). *S*, molecular weight standards: albumin (67,000), *A;* ovalbumin (45,000), *O;* chymotrypsinogen (23,000), *C;* α and β chains of rabbit hemoglobin (14,800 and 15,200), *H*.

DISCUSSION

A common feature of many nuclei is the presence of an amorphous layer of variable thickness which is apposed to the inner nuclear membranes separating it from the chromatin. This peripheral layer has been called variously the fibrous lamina (24), dense lamella (25), or the *zonula nucleum limitans* (26). Such a layer has not been observed in liver parenchymal cell nuclei *in situ* under normal fixation conditions. However, isolated rat liver nuclei that have been treated with detergent (and thus had their membranes removed) often exhibit a densely staining amorphous layer approximately 150 Å thick at the periphery of the nucleus (47–52). This layer has been mistaken for a membrane, but it has also been suggested that it may be related to the amorphous peripheral layer observed in other cell types (51).

We suspect that it is indeed likely that such a layer may not always be apparent and that the lamina demonstrated in this report is that amorphous layer. Isolation of the material from a nuclear envelope fraction, as well as the asymmetric presence and common orientation of the pore complexes on the lamina, support the idea that the lamina derives from the nuclear periphery.

Our observations are consistent with the model illustrated in Fig. 3. The inner and outer membrane are separated along their surface by the perinuclear space except at the nuclear pores, where the two membranes show a direct continuity. An amorphous lamina of varying dimensions, depending on cell

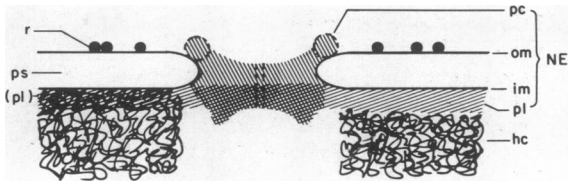

FIG. 3. Schematic diagram of the nuclear periphery (after refs. 3 and 24). Nuclear pore complex, *pc;* outer nuclear membrane, *om;* perinuclear space, *ps;* inner nuclear membrane, *im;* amorphous (peripheral) lamina, *pl;* nuclear envelope, *NE;* heterochromatin, *hc;* ribosome, *r*. The left half of the drawing indicates the appearance when the chromatin obscures the lamina.

type and perhaps metabolic state, is present immediately beneath the inner nuclear membrane. In some cell types or under certain conditions this layer may be obscured by the dense heterochromatin (see left half of figure). In such cases its presence may be indicated by an apparent thickening of the inner membrane. The nuclear pore complex fills the nuclear pore overlapping the margin of the pore on the outside and is in continuity with the amorphous lamina on the inside, as suggested by Fawcett (24).

It ought to be emphasized that ultrastructural identification at moderate resolution has been the definitive criterion during isolation of the pore complex-lamina and, thus, it is possible that there has been some loss of morphologically less important structures. It is also possible that physiologically important components have been removed. In this regard, we note that RNA accounts for only approximately 2% of the total weight of the pore complex-lamina. It has been estimated, using morphometric and chemical data obtained with manually isolated amphibian oocyte nuclear envelopes (19), that RNA should account for a large fraction by weight of the pore complex. The low level of RNA that we obtained may result from extraction during isolation or it may indicate a significant difference between pore complexes in different species.

The possibility of nonspecific adsorption of extraneous components to the pore complex-lamina seems unlikely owing to the absence of morphologically observable additions and to the paucidispersity of the polypeptides of the pore complex-lamina.

The nuclear envelope proper consists of three structures: the double membrane system, pore complexes, and a peripheral lamina. The functions of this lamina may be to provide a more or less rigid skeleton, and to spatially organize the nuclear pore complexes. The presence of a rigid lamina at the nuclear periphery may explain the fact that rat liver nuclei and many other nuclei retain their shape in the absence of a membrane. Furthermore, if the lamina does organize nuclear pore complexes, it may be responsible for the nonrandom distribution of nuclear pores in the nuclear surface (53–56). Thus, the lamina, although not always easily made visible, may be as ubiquitous a component of nuclei as the nuclear pore complexes.

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