The Triton X-100 and High Salt Resistant Residue of *Saccharomyces cerevisiae* Nuclear Membranes

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Saccharomyces cerevisiae nuclear membranes were prepared from isolated nuclei by digesting chromatin with deoxyribonuclease after an initial treatment of nuclei with very diluted buffers. When the nuclear membranes were treated with 5% Triton X-100 and 1 M NaCl an insoluble fibrous net was obtained which consisted mainly of protein with M_r values of 85000, 48000, 45000, 39000 and 31000. Lamins, a set of proteins with $M_r = 65000-75000$, which were shown to be the major proteins of the insoluble nuclear membrane residue of higher eukaryotes, were not found.

1. Introduction

There are features of *Saccharomyces cerevisiae* nuclear structure and behaviour which are rather different from those of most higher eukaryotes [1, 2]. Like in all ascomycetous yeasts and fungi the nucleus is not broken down during cell divisions. This kind of nuclear division with intact nuclear envelope probably demands specialized regions of nuclear membrane, like regions of membrane synthesis and assembly, or regions of different membrane fluidity. Other specialized regions of the nuclear envelope are visible with the electron microscope: the spindle plaques, which serve as nucleating centres for the microtubules of the intranuclear spindle. The pores of the *Saccharomyces cerevisiae* nuclear envelope seem to have a simpler structure than those of higher eukaryotes [3] and they seem to have lateral mobility, leading to uneven distribution in different stages of the cell cycle [3, 4]. It seemed possible to us that these differences reflect a molecular organization of the yeast nuclear envelope which could be different from that of higher organisms with open mitosis.

2. Experimental

2.1. Isolation of nuclear fractions

Nuclei were isolated from the haploid *Saccharomyces cerevisiae* strain SMC-19 A [5] as in [6]. The nuclear pellet was suspended in 2 mM Tris buffer, pH 7.5, containing 10% sucrose and 0.05 mM $MgCl_2$. The suspension was kept in ice for 15 min before centrifugation at $40000 \times g_{max}$ at 2 °C for 30 min. The pellet was taken up in 20 mM Tris buffer, pH 7.5, containing 0.5 mM $MgCl_2$ and 10% sucrose to obtain a suspension with 2.5–5 mg protein/ml. To this suspension 400 U of pancreatic ribonuclease and 4000 U of pancreatic deoxyribonuclease I were added per 50 mg of nuclear protein. After 90 min incubation at 22–25 °C the mixture was centrifuged as above. The pellet was suspended in the same buffer and layered onto a discontinuous sucrose gradient of 35, 42, and 52% sucrose in the same buffer. After centrifugation at $100000 \times g_{av}$ at 2 °C for 2 h the nuclear membrane fraction was removed from the 42/52% interface and diluted with 20 mM Tris buffer, pH 7.5, containing 6% Triton X-100, 1.2 M NaCl and 0.5 mM $MgCl_2$ to a final concentration of 5% Triton and 1 M NaCl. The detergent to protein ratio was usually 250–300. This suspension was gently shaken for 30 min at 22–25 °C before centrifugation at $100000 \times g_{av}$ for 30 min at 2 °C. The pellet was suspended in the usual Tris buffer and centrifuged at $40000 \times g_{max}$ for 30 min at 2 °C. 1 mM phenylmethylsulfonyl fluoride was added to all buffers shortly before use.

2.2. Analytical methods

Preparation of samples for electron microscopy was done as in [7]. Gel electrophoresis was done as in [8] with 5% stacking gel and 12% separating gel. The Triton/NaCl supernatant was first treated with

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XAD as in [9] and then dialysed against H_2O before electrophoresis. Lipids and nucleic acids were extracted as in [6]. Protein was measured as in [10] after precipitation as in [11]. Phospholipids, sterols and neutral glycerides were determined as in [12–14]. RNA was measured as in [15], and DNA was estimated by the absorbance of the hydrolysis products at 260 nm.

3. Results and Discussion

Incubation of nuclei in 2 mM Tris buffer led to bursting and extrusion of chromatin. Lumps of chromatin and ribosomes were however still bound to the membranes. They were removed with nucleases. Centrifugation of the crude membrane pellet in a continuous sucrose gradient from 30–55% sucrose gave 3 fractions. 4–8% of the total material applied to the gradient remained at the top. It contained single membrane vesicles and was very rich in neutral lipids. Another 3–5% were found in a pellet containing single membrane vesicles which were sometimes filled with residual chromatin. This fraction was rich in nucleic acids and neutral lipids. More than 80% of the total material was found in a fraction from 42–47% sucrose. The distribution of material in this fraction was not symmetrical: absorption at 600 nm was higher in the denser regions. Electron microscopic examination showed that the number of large double membrane vesicles with many pores increased with denisty. Electrophoresis showed however exactly the same protein pattern in different density regions of this fraction. Therefore it was collected as a sharp zone at the interface between 42 and 52% sucrose layers of a discontinuous gradient for the following experiments. This membrane fraction was identified as nuclear membrane fraction because it contained many double membrane profiles, either as large vesicles or as sheets, which were studded with pores (Fig. 1). Pores were much more frequent than in nuclear membrane fractions treated with high salt buffers [6, 7]. The composition of this fraction is shown in Table I. The higher recovery of nucleic acids with this isolation method as well as the presence of some strongly contrasted grains, possibly ribosomal remnants, associated with the membranes (Fig. 1 B) showed that the better preservation of nuclear envelope structural elements obtained with this mild procedure had to be payed with a higher level of impurities, compared to preparations treated with high salt solutions [6, 7].

When the nuclear membrane fraction was extracted with 5% Triton X-100 in the presence of 1 M NaCl, an insoluble residue remained, which was composed mainly of protein, but which also contained residual nucleic acids and lipids (Table I). As in other membranes [16], the neutral lipids were much more resistant to Triton extraction than phospholipids. Therefore rather high detergent concentrations had to be used. Sequential extraction, first with detergent and then with NaCl, or simultaneous extraction at 0 °C were much less effective. Electron microscopic examination of the nuclear membrane residue showed a fibrous net comparable to residues isolated from rat liver [17] or amphibian oocyte [18]. The yeast nuclear membrane residue appeared however much denser (Fig. 1 D). Structural components having the dimensions of pores were found less frequently than pores in the unextracted membranes. It is however possible that they were more difficult to identify than in higher eukaryote nuclear membrane residues because of poor preservation, lack of defined internal structures, simpler shape, or density of the surrounding

Table I. Composition and recovery of nuclear fractions. The compositon is in wt% of the sum of compounds tested. The mean values of 3 preparations are shown ± SD. Recovery values are the mean of 2 preparations.

	Nuclei	Nuclear membranes	Recovery from nuclei [%]	Insoluble residue	Recovery from membranes [%]
Protein	73.2 ± 1.9	62.1 ± 2.8	18.3	83.5 ± 2.7	8.6
Phospholipid	6.9 ± 0.6	20.9 ± 4.5	71.5	1.3 ± 0.3	0.5
Sterols	1.8 ± 0.2	5.7 ± 1.5	57.0	3.5 ± 0.9	3.5
Glycerides	1.8 ± 0.3	5.6 ± 2.1	53.0	4.0 ± 0.6	4.3
RNA	14.6 ± 1.2	4.7 ± 1.7	7.5	4.1 ± 2.1	5.2
DNA	1.9 ± 0.3	0.7 ± 0.2	6.8	4.1 ± 0.7	43.0

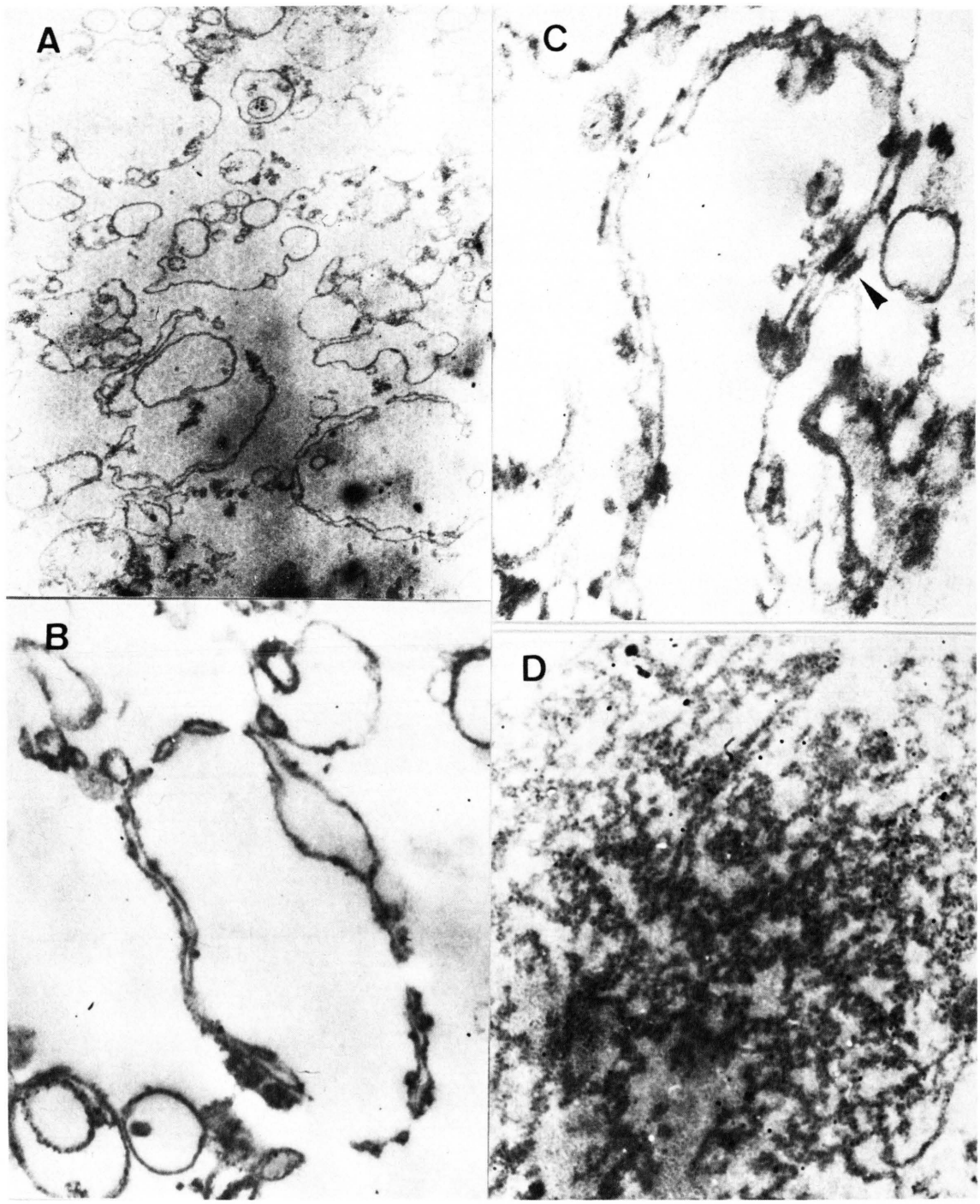

Fig. 1. (A) Isolated nuclear membranes, 30300 ×; (B) large nuclear envelope fragment with double membrane profile and pores, 47000 ×; (C) nuclear envelope fragment with pores and spindle plaque (arrow), 76000 ×; (D) the Triton X-100 and NaCl resistant membrane residue, 55500 ×.

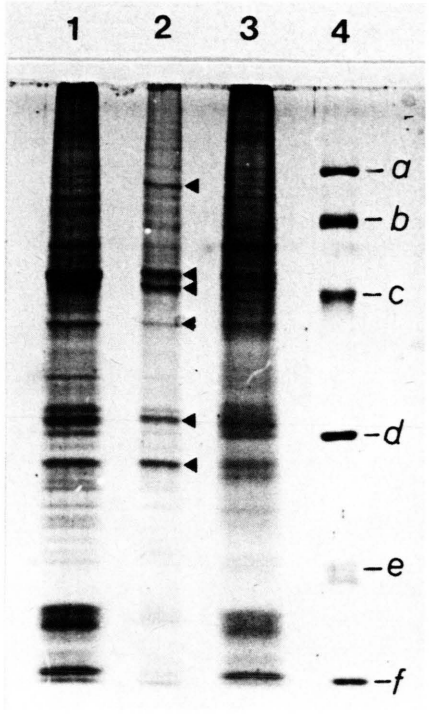

Fig. 2. Electrophoretic analysis of nuclear membrane fractions. Lane 1, nuclear membrane; lane 2, Triton and high salt resistant residue; arrows point to major proteins with M_r 85000, 48000, 45000, 39000, 31000 and 28000; lane 3, detergent and high salt soluble proteins; lane 4, M_r standards: a, phosphorylase b (94000); b, bovine albumin (67000); c, ovalbumin (43000); d, carbonic anhydrase (30000); e, trypsin inhibitor (20100); f, α-lactalbumin (14400).

fibrillar net. The polypeptide composition of the nuclear membrane fractions is shown in Fig. 2. 4 major proteins were found in the insoluble residue. They had M_r values of 48000, 45000, 31000 and 28000. Other, less prominent, but clearly enriched proteins had M_r values of 85000 and 39000. All of these proteins, except the 85 K protein, were also major proteins of the unextracted nuclear membrane (Fig. 2). Most of them were also present to a variable extent in the Triton/NaCl soluble fraction. Such a differential extraction behaviour had also been found with avian erythrocyte [19] and rat liver [20] nuclear membrane proteins. The protein pattern of the yeast nuclear membrane residue was not so simple as that of amphibian oocytes, which shows only 1 major protein with $M_r = 68000$ [21]. The protein pattern of rat liver nuclear membrane residue, on the other hand, is dominated by a triplet of proteins with $M_r = 65000-75000$ [17]. These proteins, the lamins, were shown to be the major proteins of the lamina, a protein structure subjacent to the inner nuclear membrane in intact envelopes. During mitosis, when the nuclear envelope of higher eukaryotes is broken down, the lamina is depolymerized [22]. Judged from the M_r of the major proteins, lamins, if present at all, seem not to play a role in the insoluble residue of the yeast nuclear membrane. At present we do not know whether other proteins replace them, or whether the yeast nuclear envelope has a different structural organization. Furthermore it should be stressed, that the yeast nuclear membrane residue was isolated from rapidly dividing cells. Therefore it is still possible that nuclear membrane residues from resting yeast cells will show more resemblance to the pore complex-laminae of higher eukaryotes.

Acknowledgements

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