# ISOLATION AND CHARACTERIZATION OF NUCLEI AND NUCLEAR MEMBRANES FROM *SACCHAROMYCES CEREVISIAE* PROTOPLASTS

K. MANN and D. MECKE

*Physiologisch-chemisches Institut der Universität Tübingen, Hoppe-Seyler-Straße 1, D-7400 Tübingen, FRG*

Received 23 October 1980

## 1. Introduction

The nuclei of ascomycetous yeasts differ in several respects from nuclei of most other eukaryotes. The most obvious differences are the absence of nuclear envelope disintegration during cell divisions and an intranuclear spindle built up with microtubules which arise from specialized regions of the envelope, the centriolar plaques [1,2]. We think that these differences should be reflected in the composition and molecular organization of the nuclear envelope. Therefore we have isolated the yeast nuclear membranes and determined their chemical composition and electrophoretic protein pattern as a first step to the detection and understanding of such differences.

## 2. Experimental

### 2.1. *Isolation of nuclei and nuclear membranes*

A haploid yeast strain (SMC-19 A) and a diploid strain (D.19) derived from the haploid one by mating with another haploid of opposite mating type (cat 1.S3-14 A) were used for isolation of nuclei [3]. The ability of diploids to sporulate was used as control for the success of mating. The cells were grown in a medium containing 1% yeast extract, 2% casein peptone, and 4% glucose at 30°C with continuous shaking. They were harvested in the log-phase of growth by centrifugation at 3000 × $g_{av}$, washed 2× with water, and then suspended in a 10 mM phosphate buffer (pH 6.5) containing 1.3 M sorbitol. This suspension was incubated 30 min at 30°C with 10–13 mg zymolyase 5000 (Kirin Brewery, Japan)/g pelleted cells. The resulting protoplasts were washed 2× in the sorbitol medium.

For isolation of nuclei the protoplasts were suspended in the Ficoll medium of [4] i.e., 0.02 M phosphate buffer (pH 6.5), 0.5 mM $MgCl_2$, and 18% Ficoll. This suspension was homogenized with a Potter-Elvehjem homogenizer at 800 rev./min. The nuclei were always kept near 0°C. The suspension was centrifuged at 5000 × $g_{max}$ for 15 min, the pellet discarded, and the supernatant centrifuged once again at 25 000 × $g_{max}$ for 30 min. The pellet of nuclei was suspended in 0.02 M phosphate buffer (pH 6.5), 1 mM $MgCl_2$ and 20% Ficoll. This suspension was layered onto a cushion of 50% Ficoll in the same buffer, and the nuclei were pelleted at 100 000 × $g_{av}$ for 90 min in a Beckman SW 65 Ti rotor.

For isolation of nuclear membranes the nuclear pellet was suspended with a Dounce homogenizer in the citrate containing buffer of [5] i.e., 0.05 M Tris (pH 7.5), 0.025 M KCl, 0.005 M $MgCl_2$ and 10% $K_3$-citrate. This suspension was sonicated 3 × 5 s with 15 s intervals with a Branson B-12 sonifier at setting 4. The suspension was then layered onto a discontinuous gradient of 20, 25, 30 and 35% Ficoll in the same buffer, and centrifuged at 150 000 × $g_{av}$ for 2 h. The bands were removed with a pipette, diluted with the Tris–citrate buffer, and centrifuged for 45 min at 100 000 × $g_{av}$. The pellets were suspended once again with a Dounce homogenizer in the Tris–citrate buffer and centrifuged as before. All operations were carried out near 0°C. In some preparations 1 mM PMSF (phenylmethylsulfonylfluoride) was included in all media from protoplast lysis on.

### 2.2. *Analytical methods*

For electron microscopy, nuclear pellets were fixed with 5% glutaraldehyde in 20% Ficoll medium at 4°C overnight. Membranes were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Prepara-

tions were then post-fixed in 1% $OsO_4$, contrasted with 1% uranyl acetate, and embedded in the ERL medium of [6].

For chemical analyses lipids were extracted according to [7]. Part of the extract was assayed for sterols as in [8] with ergosterol as standard. Another part was hydrolysed in 0.5 M KOH–95% ethanol and freed glycerol was tested according to [9] with triolein standards. A third part of extract was hydrolysed in 10 N $H_2SO_4$ at 160°C overnight and tested for phosphorus according to [10]. RNA was extracted with 1 N NaOH at 37°C and assayed with the orcinol method [11]. DNA was extracted with 0.5 N $HClO_4$ at 80°C and assayed with diphenylamine [12]. Yeast RNA and calf thymus DNA were used as standards. Protein was assayed before extraction of other compounds according to [13].

Electrophoreses were done according to [14] with 4% stacking gel and 12% seperating gel. Gels were stained for glycoproteins as in [15].

## 3. Results and discussion

### 3.1. *Nuclei*

Yeast nuclei were already isolated from several species [4,16–19], but the lipid content was never tested. We tried the method in [4] for *Saccharomyces fragilis* protoplasts, but the nuclei we got were heavily contaminated with small vacuolar vesicles which could however be eliminated by an additional centrifugation and an increase in density in the last centrifugation step (30–50% Ficoll). A large part of the nuclei was undamaged and showed a closed nuclear envelope with an outside covered by ribosomes (fig.1,2). Nuclear pores were only seen when the perinuclear cistern was

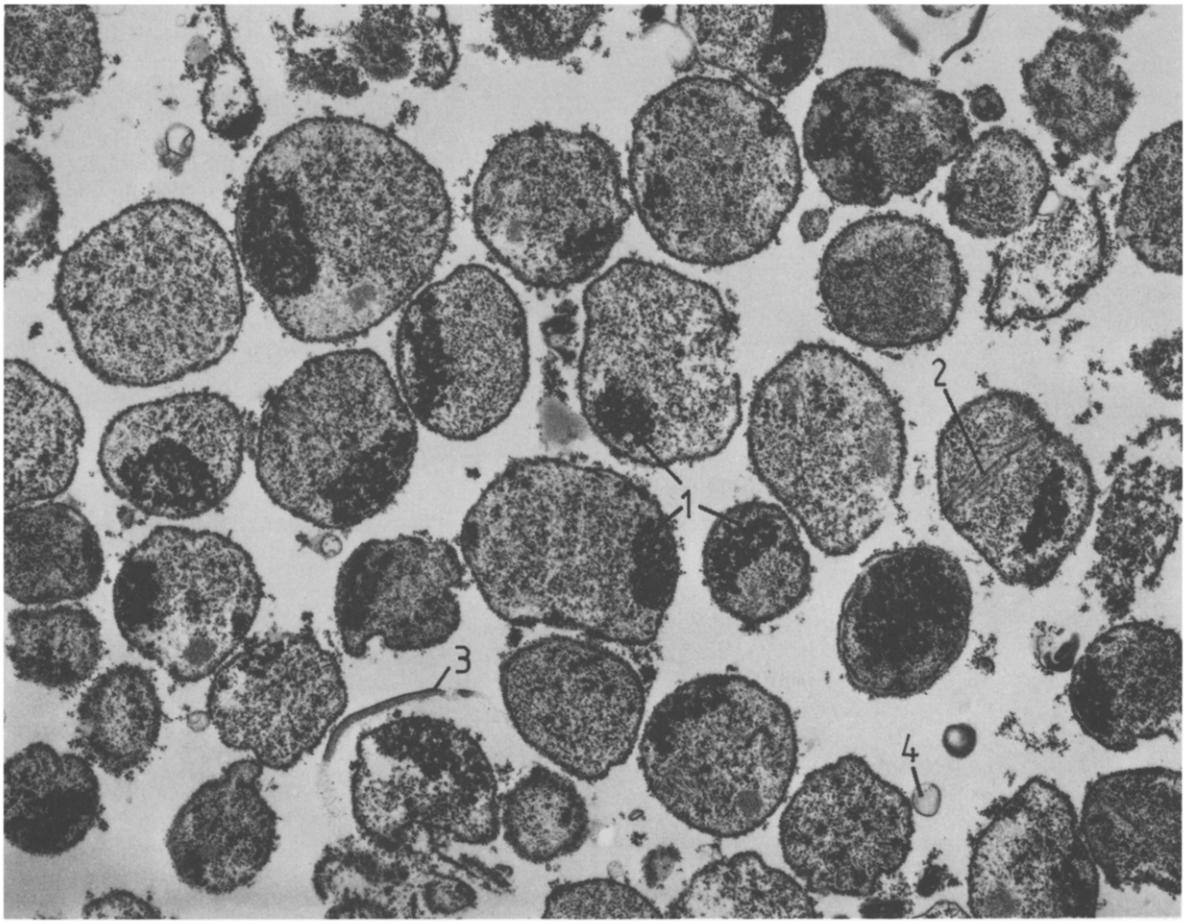


Fig.1. Haploid nuclei 14 000×: 1, dense crescent; 2, spindle; 3, piece of undigested cell wall, 4, smooth membrane vesicle.

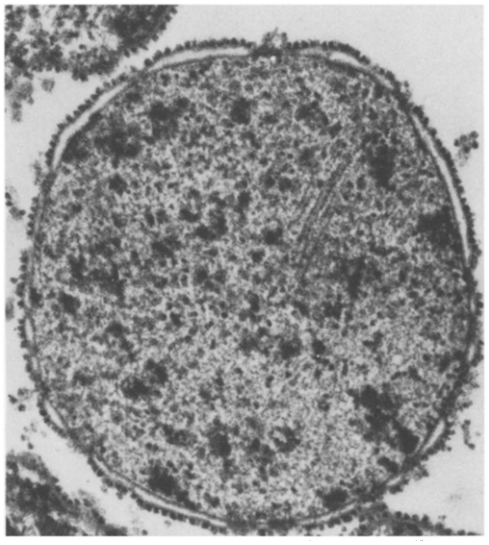

Fig.2. Nucleus with clearly visible perinuclear cistern and pore complexes: 46 000×.

a little swollen (fig.2). But usually the clearly discernible 2 membranes were closely together. Often spindle microtubules and dense crescent (nucleolus) were seen (fig.1,3). The nuclei were contaminated by few smooth membrane vesicles, pieces of undigested cell wall (which disappear if more zymolyase is added), and by some short pieces of endoplasmatic reticulum which were rarely standing out from the nuclear surface. Chromatin from bursted nuclei was sometimes trapped between whole nuclei. As usual for yeast nuclei the RNA content was very high, while DNA was only a smaller fraction of the tested compounds (table 1). Protein and lipid content were comparable to nuclei from higher eukaryotes [5,20,21]. Haploid and diploid nuclei showed only little difference in relative composition and ultrastructure. The yield was ~30% (DNA recovery).

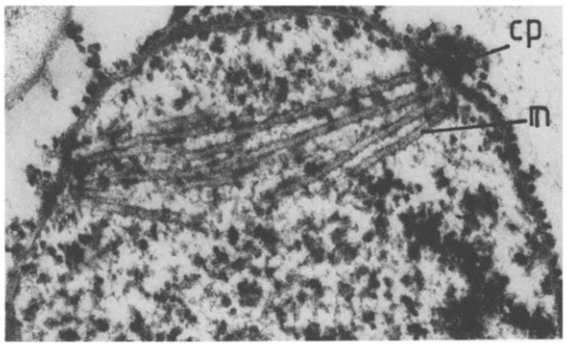


Fig.3. Spindle 56 000×: cp, centriolar plaque; m, microtubule.

Table 1
Composition of nuclei and nuclear membranes in wt% of the sum of compounds tested

| | Diploid nuclei | Haploid nuclei | Nuclear membranes |
|---|---|---|---|
| Protein | 73.5 ± 0.7 | 73.2 ± 1.9 | 50.5 ± 2.6 |
| Phospholipid | 6.2 ± 1.3 | 6.9 ± 0.6 | 31.8 ± 1.4 |
| Sterols | 1.7 ± 0.2 | 1.8 ± 0.2 | 5.0 ± 0.7 |
| Glycerides | 1.9 ± 0.3 | 1.8 ± 0.3 | 4.3 ± 0.4 |
| RNA | 14.8 ± 1.2 | 14.6 ± 1.2 | 7.9 ± 0.6 |
| DNA | 1.9 ± 0.3 | 1.7 ± 0.2 | 0.5 ± 0.15 |

The mean values of 4 preps. of each type of nucleus and of 3 preps. of nuclear membrane are shown ±SD. Nuclear membrane was prepared only from haploid nuclei

### 3.2. *Nuclear membranes*

Nuclear membranes were for the present isolated from haploid nuclei only. Centrifugation of nuclear lysates in the Ficoll gradient yielded 4 fractions and a small pellet of residual nuclei (fig.4). Fractions 1 and 2 showed small single membrane vesicles and dispersed residual chromatin fibres. ~40% of the material was nucleic acid. >80% of the nuclear lysate was present in fraction 3, consisting of vesicles of all sizes, some reaching even the size of whole nuclei (fig.5). As with nuclei, 2 closely approaching membranes were often visible, but pores could not be identified with certainty. Compared with nuclear membranes from higher

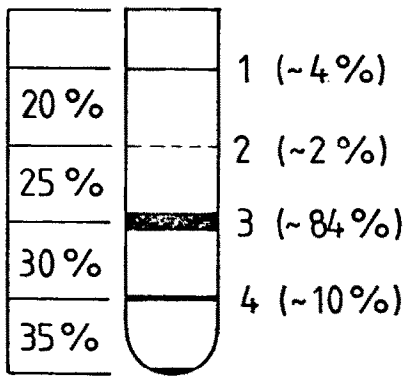


Fig.4. Separation of lysed nuclei fractions in a discontinuous Ficoll gradient; left of the tube the Ficoll concentration is shown, on the right the fractions are numbered as in the text, and the part of total banded material found in each single band is given in brackets.

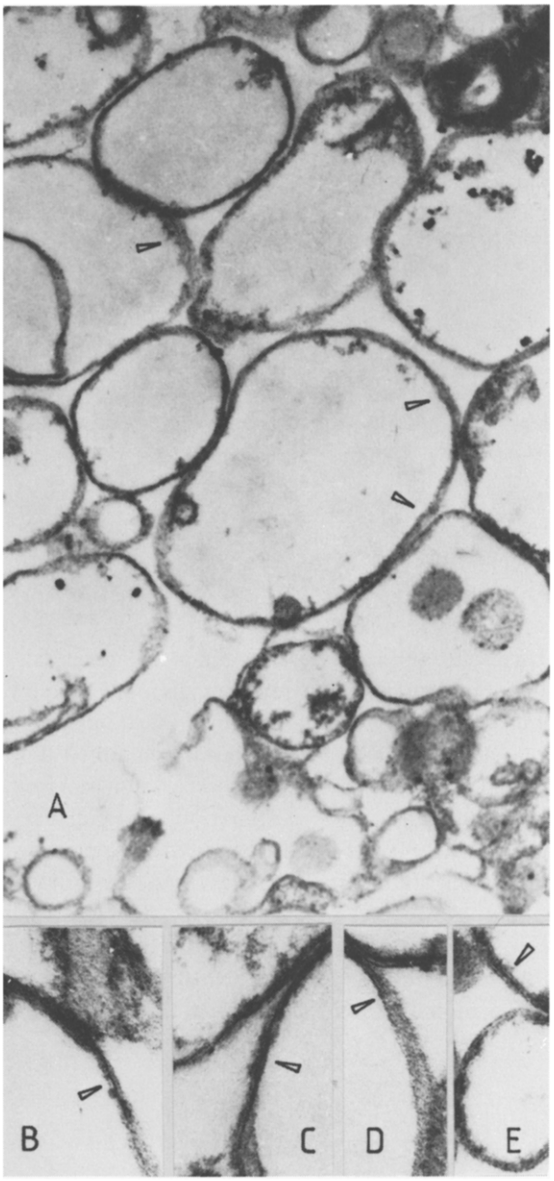


Fig.5. Nuclear membranes from fraction 3; (A) 36 000×; (B–E) 82 800×; some double membrane structures are marked with an arrow.

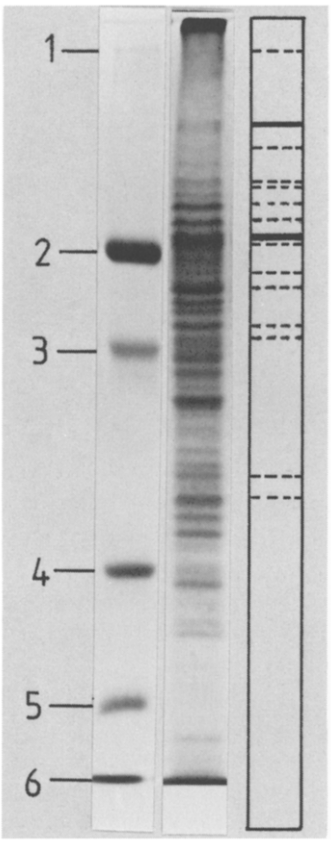


Fig.6. Electrophoretic analysis of nuclear membrane proteins. Left, $M_r$ standards: 1, albumin dimer (134 000); 2, albumin (67 000); 3, ovalbumin (45 000); 4, trypsinogen (24 000); 5, lactoglobulin (18 400); 6, lysozyme (13 400); right, positions of PAS-stainable bands.

eukaryotes, this membrane contained ~10% less protein and relatively more lipid (table 1). The RNA content is comparable to other preparations, while there is less DNA than in most other nuclear membranes [20–23]. Only in [5] was no DNA found, but their membranes were kept for ~24 h in the 10% citrate buffer. The protein pattern of this fraction was rather complicated (fig.6). There remained some insoluble material at the top of the gel, but no protein with $M_r \gtrsim 130\,000$ was detected even in gels with 8% polyacrylamide. Addition of 1 mM PMSF from lysis of protoplasts up to denaturation of samples for electrophoresis did not change this pattern or the chemical composition of the membranes. With PAS stain for sugars, 2 major bands with $M_r$ ~105 000 and ~73 000 were detected in the gels. Traces of bands were found in other parts of the gel (fig.6). Fraction 4 of the Ficoll gradient showed nuclear membrane vesicles with lumps of chromatin inside. We did not try to isolate pure membrane from this fraction because it did not contain enough material and because the protein pattern in electrophoresis showed no significant differences to that from fraction 3.

From the much larger nuclei of animal liver or higher plant tissues large sheets of membrane were

often isolated, showing more or less clearly the pore complexes [5,20,21]. Strong sonication of these nuclei resulted in loss of pore material and formation of small single membrane vesicles. It is true that our preparations showed more vesicles than sheets, but these vesicles were often ⩾50% as large as whole nuclei. They also often showed the same compressed double membrane system found in our nuclei preparation and others. Reduction of sonication to 3 × 2 s did not change the appearance of vesicles, but nucleic acid content was doubled. We hope that the pores will become more clearly visible, when fractionating the nuclear membranes described here.

## Acknowledgements

We thank Professor Oberwinkler for placing his electron microscope at our disposal, Miss S. Dinkelmeyer for operating the microscope, and Dr K. D. Entian for supplying us with the yeast strains.

## References

[1] Hartwell, L. H. (1974) Bacteriol. Rev. 38, 164–198.
[2] Carter, B. L. A. (1978) Adv. Microbiol. Physiol. 17, 243–302.
[3] Entian, K. D. (1980) Mol. Gen. Genet. 178, 633–637.
[4] May, R. (1971) Z. Allgem. Mikrobiol. 11, 131–142.
[5] Kashnig, D. M. and Kasper, C. B. (1969) J. Biol. Chem. 244, 3786–3792.
[6] Spurr, A. R. (1969) J. Ultrastruct. Res. 26, 31–43.
[7] Folch, J., Lees, M. and Sloane Stanley, G. H. (1957) J. Biol. Chem. 226, 497–509.
[8] Glick, D., Fell, B. F. and Sjølin, K. E. (1964) Anal. Chem. 36, 1119–1121.
[9] Pinter, J. K., Hayashi, J. A. and Watson, J. A. (1967) Arch. Biochem. Biophys. 121, 404–414.
[10] LeBel, D., Poirier, G. G. and Beaudoin, A. R. (1978) Anal. Biochem. 85, 86–89.
[11] SanLin, R. J. and Schjeide, O. A. (1969) Anal. Biochem. 27, 473–483.
[12] Burton, K. (1956) Biochem. J. 62, 315–323.
[13] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[14] Laemmli, U. K. (1970) Nature 227, 680–685.
[15] Zacharius, R. M., Zell, T. E., Morrison, J. H. and Woddlock, J. J. (1969) Anal. Biochem. 30, 148–154.
[16] Duffus, J. H. (1969) Biochim. Biophys. Acta 195, 230–233.
[17] Molenaar, J., Sillevis Smitt, W. W., Rozijn, T. H. and Tonino, G. J. (1970) Exp. Cell Res. 60, 148–156.
[18] Bhargava, M. M. and Halvorson, H. O. (1971) J. Cell Biol. 49, 423–429.
[19] Wintersberger, U., Smith, P. and Lenansky, K. (1973) Eur. J. Biochem. 33, 123–130.
[20] Kartenbeck, J., Jarasch, E. D. and Franke, W. W. (1973) Exp. Cell Res. 81, 175–194.
[21] Philipp, E. J., Franke, W. W., Keenan, T. W., Stadler, J. and Jarasch, E. D. (1976) J. Cell Biol. 68, 11–29.
[22] Stavy, R., Ben-Shaul, Y. and Galun, E. (1973) Biochim. Biophys. Acta 323, 167–177.
[23] Shelton, K. R., Cobbs, C. S., Povlishock, J. T. and Burkat, R. K. (1976) Arch. Biochem. Biophys. 174–186.