ISOLATION AND CHARACTERIZATION OF NUCLEAR ENVELOPE FRAGMENTS FROM *DICTYOSTELIUM*

Rosmarie WIDMER and Roger W. PARISH

Cytology, Plant Biology Institute, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

Received 22 September 1980

1. Introduction

Although the nuclear envelope is presumably involved in the specific nucleocytoplasmic exchange of macromolecules, in particular RNA and proteins, its precise functional role in the cell is still unknown [1–4]. The cellular slime mold *Dictyostelium discoideum* is a primitive eukaryote with a genome only ~11-times the size of the *E. coli* genome [5]. Morphogenetic changes occurring during the life cycle are accompanied by modulations of gene activity [6]. Consequently, we were interested to isolate and characterize the nuclear envelope with a view to examining any compositional changes occurring during differentiation.

Heparin and DNase I treatment followed by sucrose density gradient centrifugation were used to isolate nuclear envelope fragments from *D. discoideum* amoebae. Typical nuclear pore complexes were observed. Low Mg^{2+}-ATPase activity was found, but alkaline phosphatase, acid phosphatase, cyclic AMP phosphodiesterase and NADH-cytochrome *c* reductase activities were not detectable. Six major proteins were found on SDS gels stained with Coomassie blue, one of which (68 kM_r) was a glycoprotein. No group of proteins was found corresponding to the 3 major polypeptides (60–70 kM_r) believed associated with the lamina and consistently present in nuclear envelopes of higher eukaryotes. A large number of glycoproteins (>40) were detected. The majority of these glycoproteins were also present in the plasma membrane although, with 4 exceptions, at different concentrations. All glycoproteins detected in intact nuclei were represented in the nuclear envelope.

Abbreviations: con A, concanavalin A; kM_r, $\times 10^3$ relative molecular mass; SDS–PAGE, sodium dodecylsulphate–polyacrylamide gel electrophoresis

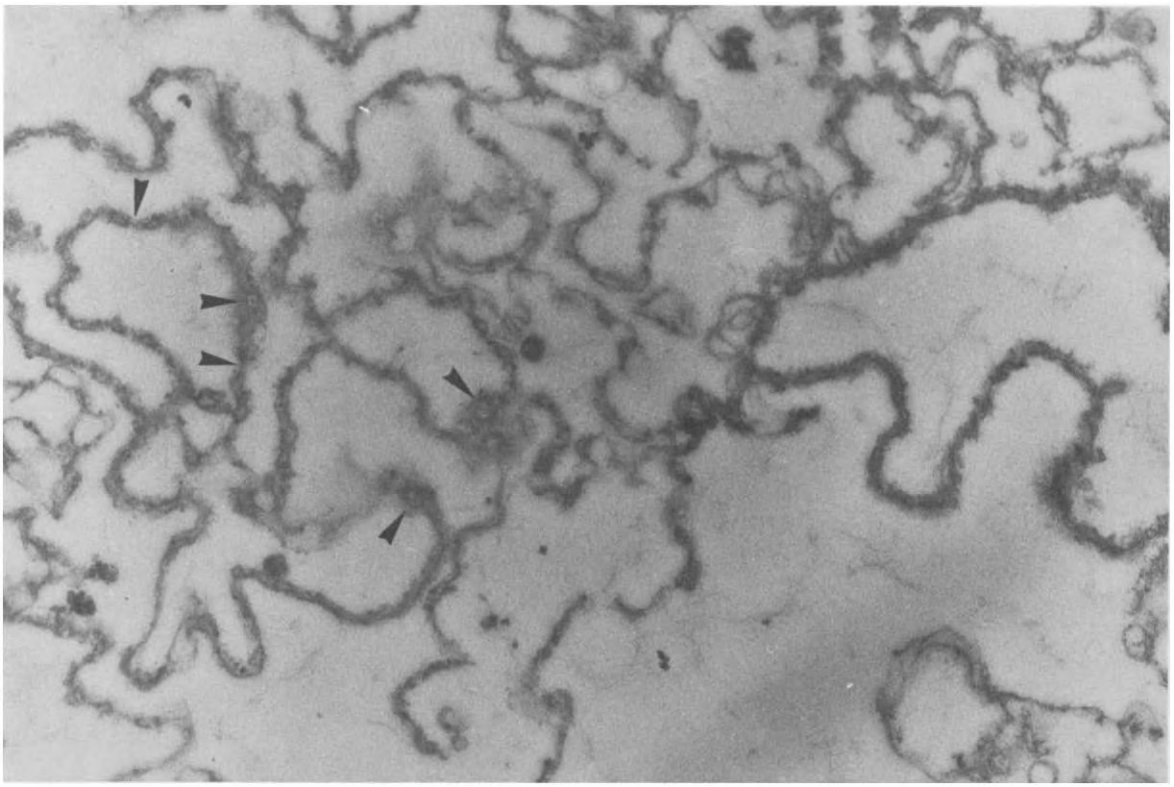
2. Materials and methods

Dictyostelium discoideum cells (strain Ax-3) were grown in HL-5 medium [7]. Cells were harvested at the end of the exponential growth phase (5–6 × 10^7 cells/ml). Nuclei were isolated as in [8].

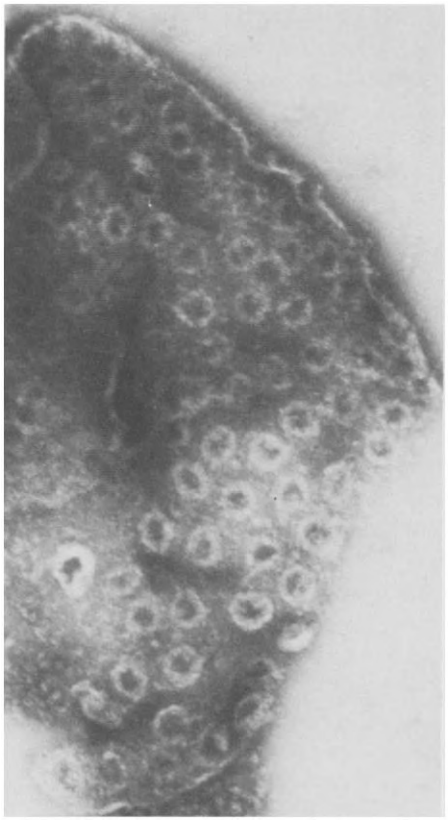
The method we used to isolate nuclear envelope was based on 3 different techniques [4,9–11]. Nuclei were suspended in 10 mM Tris–HCl (pH 7.5), 10 mM Na_2HPO_4 to 100 μg DNA/ml. Heparin (stock solution 100 mg/ml) was added (1 μl/ml suspension) and the solution gently shaken. The suspension became viscous within a few seconds and subsequently 3 μg DNase I (2465 U/mg, RNase-free, Worthington) were added/ml suspension. Following incubation for 20 min at 23°C, the suspension was placed in ice and 1/3 vol. 1 M sucrose added. The suspension was loaded onto a sucrose step gradient (2.2, 2.0, 1.8 and 1.5 M sucrose in 10 mM Tris–HCl, pH 7.5) and centrifuged at 100 000 × *g* for 90 min. The nuclear envelopes were found at 1.5–1.8 M sucrose. The membrane band was collected, diluted with 1 vol. 10 mM Tris–HCl (pH 7.5) and centrifuged at 100 000 × *g* for 30 min.

We tested a number of other methods in an attempt to isolate nuclear envelopes. These included heparin [10], $NaHCO_3$ and DNase I [4], high salt concentrations [11,12] and a combination of high salt and DNase I [13]. None of these methods gave clean nuclear envelopes. A combination of heparin and DNase I has also been used in [36].

The enzymes NADH-cytochrome *c* reductase, alkaline and acid phosphatase were measured as in [14–16]. Cyclic AMP phosphodiesterase and ATPase were measured using radioactive [^{14}C]substrates, isolating the products by thin-layer chromatography and measuring their radioactivity.



a



b

3. Results and discussion

The solubilization of nuclear contents following addition of heparin could readily be observed in the phase contrast microscope. After 1–2 min nuclear envelopes (ghosts) were seen. An envelope preparation was only obtained when dilute suspensions of nuclei were used (100 μg DNA/ml) and the DNA: heparin ratio was 1:1. At higher heparin concentrations the ghosts disintegrated while at lower concentrations the nuclear contents were inadequately solubilized. When ghosts were examined by electron microscopy fibrillar material was observed attached to the membranes. This was removed by the DNase I (fig.1). If DNase I treatment was inadequate or omitted, the nuclear envelopes no longer banded between

Fig.1. (a) Thin-section of purified nuclear envelopes (×31 500). Regions sectioned perpendicular to the plane of the envelope show the presence of inner and outer nuclear membrane and pore complexes (arrows). Regions sectioned parallel to the envelope also show pore complexes (arrows), ribosome particles are not present. (b) Nuclear envelope, negatively stained with sodium phosphotungstate, showing nuclear pore complexes (×44 000).

1.5 and 1.8 M sucrose in gradients but were found at higher densities or in the sediment. SDS–PAGE of such preparations gave protein patterns similar to whole nuclei (not shown). The various other methods tested invariably resulted in envelope fractions containing considerable amounts of histones (fig.2a).

Thin sectioning indicated fairly large sheets of torn nuclear envelopes were present (fig.1a). Both the inner and the outer nuclear membranes could be observed in places. Nuclear pore complexes were present. Negative staining (fig.1b) indicated the dimensions of these complexes (~65 and 100 nm for inner and outer diameters, respectively) resembled those of higher eukaryotes [4]. The majority of nuclear pore complexes did not have a central granule. Few ribosome particles were observed.

Except for low Mg^{2+}-ATPase activity (4 nmol ATP . mg protein^{-1} . min^{-1}) none of the other enzymes sought could be detected. We thought that Triton X-100 (used for the isolation of nuclei) or heparin might reduce enzyme activities. However, the activities of ATPase, cyclic AMP phosphodiesterase, acid and alkaline phosphatase in plasma membranes were not affected by similar treatments. The NADH-cytochrome *c* reductase activity is in any case low in *D. discoideum* cells [16,17]. Whole nuclei also contained barely detectable activities of these enzymes and the absence of such 'typical' nuclear envelope enzymes [3,4] is puzzling.

SDS–PAGE of purified nuclear envelopes identified 6 proteins staining strongly with Coomassie blue (210, 68, 54, 42, 27 and 26 kM_r) (fig.2b,c). A considerable number of weaker protein bands were also observed. (Note: although the presence of Triton X-100 during nuclear isolation did not lead to loss of the outer nuclear membrane, selective solubilization of some proteins cannot be excluded.) No low M_r basic proteins originating from ribosomes were detected, confirming the electron microscopic results. Any ribosomes present were apparently lost during envelope isolation. The 210 and 42 kM_r proteins were myosin heavy chains and actin, respectively. Micro-

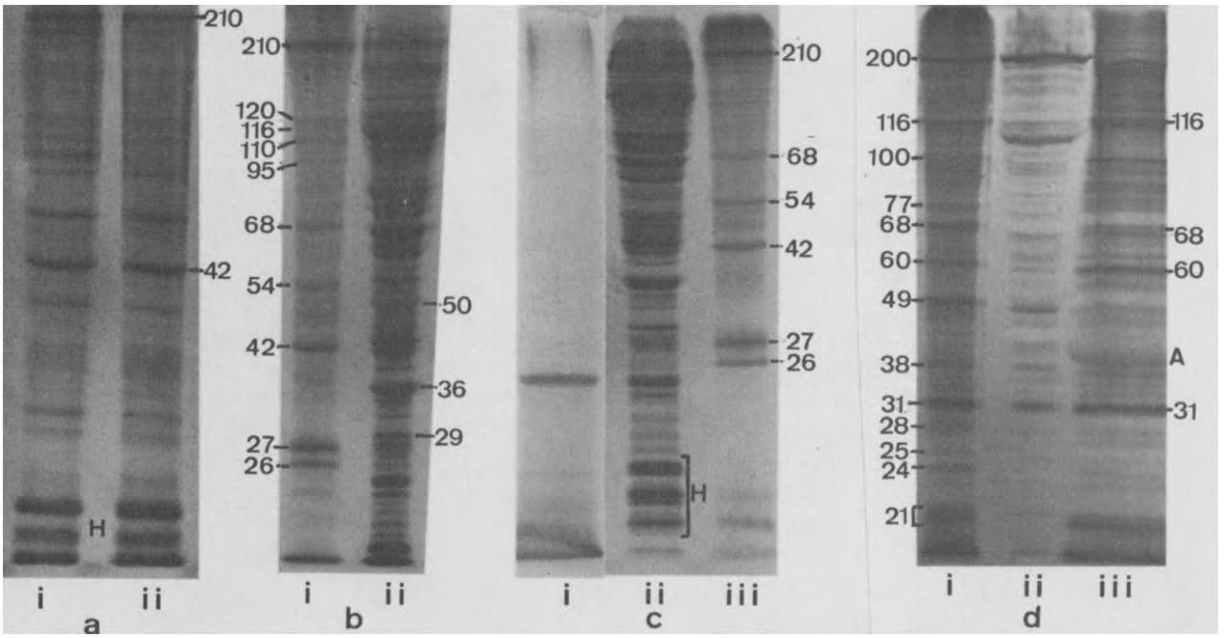


Fig.2. SDS–PAGE [20] of whole nuclei, nuclear envelope fractions, histone H1 and plasma membranes: (a–c) stained with Coomassie blue; (d) stained with con A/peroxidase [21]. kM_r values are indicated. (a) Nuclear envelope fractions isolated according to [4] = (i) and [2] = (ii) and run on an ~11% gel (H = core histones); (b) 10% gel of (i) nuclear envelope, (ii) whole nuclei; (c) 12% gel of (i) histone H1 isolated according to [8], (ii) whole nuclei (H = core histones), (iii) nuclear envelope (on higher % gels the two lowest bands did not comigrate with histones; (d) 10% gel showing glycoproteins present in (i) nuclear envelope, (ii) whole nuclei (bands slightly distorted towards lower M_r due to large amounts of protein loaded), (iii) plasma membrane (isolated according to [32]); A, indicates position of actin which is not stained but distorts the neighbouring glycoprotein bands.

filaments have been found in nuclei of *D. discoideum* [18] as well as in the cytoplasm and attached to the plasma membrane [19]. They may be attached to both surfaces of the nuclear envelope.

In higher eukaryotes 3 prominent polypeptides, which migrate between 60–70 kM_r on SDS gels, are found in the pore complex–lamina fraction [13,22–25]. Electron microscopy immunological techniques indicate these proteins are constituents of the distinct electron-dense layer (lamina) interposed between the inner nuclear membrane and the peripheral chromatin elements rather than structural elements of the nuclear pore [24,26]. In *D. discoideum* nuclear envelopes only one major protein was found in this M_r range (68 kM_r) and was a glycoprotein (fig.2b–d). Hence, the lamina was either lost during envelope isolation (proteins with the relevant sizes were major components of whole nuclei) or contains different proteins in lower eukaryotes (e.g., 26 and 27 kM_r).

The nuclear envelope contained an unexpectedly large number of glycoproteins (fig.2d). Over 40 bands were detected with the con A/peroxidase technique, 25% of which stained strongly. The majority of these were barely detectable on Coomassie blue-stained gels. A notable exception was the 68 kM_r protein. In higher eukaryotes a 68 kM_r nuclear envelope protein is preferentially phosphorylated by an intrinsic protein kinase [27]. No glycoproteins were found in whole nuclei which did not also occur in the nuclear envelope (fig.2d). This is interesting since there are reports of glycoproteins directly associated with chromatin [28,29].

Except for myosin heavy chains and actin no clear correspondence between plasma membrane and nuclear envelope proteins was observed in Coomassie blue-stained gels (not shown). This was not the case for glycoproteins. Although the relative concentrations were very different [30] the majority of nuclear envelope glycoproteins were also present in plasma membranes. (Comparisons based on M_r.) Four glycoproteins (116, 100, 60 and 31 kM_r) were major constituents of the plasma membrane and the nuclear envelope.

The similarity in glycoprotein content between nuclear envelope and plasma membrane may reflect their common origin in the ER. (The relationship between glycoproteins of the ER and nuclear envelope could not be ascertained as no method exists for the isolation of pure ER from *D. discoideum*.) Significant differences have been described in calf thymocytes [30]. Con A binds to both the inner and outer nuclear membranes of higher eukaryotes [31]. The function of membrane glycoproteins is largely unknown, although some presumably function as receptors or are involved in transport. Pore complexes need not constitute the sole passageway of molecular movement between the nucleus and the cytoplasm. The developmentally-regulated plasma membrane glycoproteins [32], some of which have been implicated in cellular adhesion [33–35], are not found in the nuclear membranes of differentiating cells (unpublished). Specific nuclear glycoproteins are synthesized during *D. discoideum* differentiation (unpublished) and it will be interesting to relate changes in nuclear envelope composition to differentiation.

Acknowledgements

We thank Peter Gehrig for the electron microscopy. This work was supported by a grant from the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung (grant no. 3.421-0.78).

References

[1] Kessel, R. G. (1973) Prog. Surface Membrane Sci. 6, 243–329.
[2] Franke, W. W. (1974) Int. Rev. Cytol. Suppl. 4, 71–236.
[3] Franke, W. W. (1974) Phil. Trans. Roy. Soc. Lond. B 268, 67–93.
[4] Harris, J. R. (1978) Biochim. Biophys. Acta 515, 55–104.
[5] Katz, E. R. (1978) Bioscience 28, 692–697.
[6] Firtel, R. A. (1972) J. Mol. Biol. 66, 363–377.
[7] Cocucci, S. M. and Sussman, M. (1970) J. Cell Biol. 45, 399–407.
[8] Charlesworth, M. C. and Parish, R. W. (1977) Eur. J. Biochem. 75, 241–250.
[9] Hildebrand, C. E. and Okinaka, R. T. (1976) Anal. Biochem. 75, 290–300.
[10] Bornens, M. (1977) Nature 270, 80–82.
[11] Frank, W. W. (1974) in: Biochemische Cytologie der Pflanzenzelle (Jakobi, G. ed) pp. 15–40, G. Thieme Verlag, Stuttgart.
[12] Philipp, E. I., Franke, W. W., Keenen, T. W., Stadler, J. and Janasch, E. D. (1976) J. Cell Biol. 68, 11–29.
[13] Jackson, R. C. (1976) Biochemistry 15, 5641–5651.
[14] Parish, R. W. and Pelli, C. (1974) FEBS Lett. 48, 293–296.
[15] Parish, R. W. (1975) Planta 123, 15–31.

[16] Parish, R. W. (1975) Eur. J. Biochem. 58, 523–531.
[17] Hintermann, R. and Parish, R. W. (1979) Exp. Cell Res. 123, 429–434.
[18] Fukui, Y. (1978) J. Cell Biol. 76, 146–157.
[19] Clarke, M., Schatten, G., Mazia, D. and Spudich, J. A. (1975) Proc. Natl. Acad. Sci. USA 72, 1758–1762.
[20] Laemmli, U. K. (1970) Nature 227, 680–685.
[21] Parish, R. W., Schmidlin, S. and Müller, U. (1977) Exp. Cell Res. 110, 267–276.
[22] Bornens, M. and Kasper, C. B. (1973) J. Biol. Chem. 248, 571–579.
[23] Krohne, G., Franke, W. W. and Scheer, U. (1978) Exp. Cell Res. 116, 85–102.
[24] Gerace, L., Blum, A. and Blobel, G. (1978) J. Cell Biol. 79, 546–566.
[25] Lam, K. S. and Kasper, C. B. (1979) J. Biol. Chem. 254, 11713–11720.
[26] Krohne, G., Franke, W. W., Ely, S., D'Arcy, A. and Jost, E. (1978) Cytobiologie 18, 22–38.
[27] Lam, K. S. and Kasper, C. B. (1979) Biochemistry 18, 307–311.
[28] Rizzo, W. B. and Bustin, M. (1977) J. Biol. Chem. 252, 7062–7067.
[29] Kurth, P. D., Bustin, M. and Moudrianakis, E. N. (1979) Nature 279, 448–450.
[30] Monneron, A. and d'Alayer, J. (1978) J. Cell Biol. 77, 232–245.
[31] Monneron, A. and Segretain, D. (1974) FEBS Lett. 42, 209–213.
[32] Parish, R. W. and Schmidlin, S. (1979) FEBS Lett. 98, 251–256.
[33] Müller, K. and Gerisch, G. (1978) Nature 274, 445–449.
[34] Geltosky, J. E., Weseman, J., Bakke, A. and Lerner, R. A. (1979) Cell 18, 391–398.
[35] Steinemann, C. and Parish, R. W. (1980) Nature 286, 621–623.
[36] Harris, J. R. (1979) J. Cell Sci. 34, 81–90.