ON THE ATTACHMENT OF THE NUCLEAR PORE COMPLEX

ROBERT PETER AARONSON and GÜNTER BLOBEL

From The Rockefeller University, New York 10021

ABSTRACT

Electron microscope examination of isolated rat liver nuclei after treatment with the detergent Triton X-100 revealed the complete removal of both the inner and outer membranes of the nuclear envelope. The envelope-denuded nuclei did not show any change in either shape or internal ultrastructure. Most strikingly, the nuclear pore complexes, which in untreated nuclei appear to be integral components of the nuclear envelope, were retained in their characteristic location at the distal ends of the channels leading through the peripheral heterochromatin.

Determination of the chemical composition of detergent-treated nuclei showed that over 95% of the nuclear phospholipid was solubilized, thus corroborating the morphological absence of nuclear membranes. Furthermore, detergent treatment also solubilized approximately 10% of the nuclear protein. Analysis of the solubilized protein by polyacrylamide gel electrophoresis in the presence of SDS indicated that these proteins belong to a few specific classes which presumably represent the major polypeptides of the nuclear membranes.

The total absence of the nuclear envelope on both morphological and biochemical grounds supports the idea that the nuclear pore complex does not require the membranes either for attachment to the nucleus or for maintenance of its own structural integrity.

INTRODUCTION

In eukaryotes, the nuclear pore complex is a ubiquitous organelle situated within the characteristic circular discontinuities ("pores") of the nuclear envelope (1-9). The roles which have been postulated for the pore and the complex fall primarily into two mutually compatible classes: (a) involvement in nucleo-cytoplasmic communication (9-12), and (b) organization of the interphase chromatin (13-16). Little direct evidence has been obtained to support either role, and there is only fragmentary information concerning the chemical composition of the complex (17, 18).

Before attempting the isolation and chemical characterization of the pore complex, it was necessary to determine the structural relationships between the pore complex and its surrounding nuclear membranes and between the pore complex and the underlying peripheral chromatin.

Nuclear pore complexes have been described in nuclear envelope fractions (19-26). Their presence suggests that the bulk of the peripheral chromatin is not necessary for maintaining the gross structural integrity of the complex. However, significant amounts of chromatin are routinely recovered.
in such fractions, so one can not rule out the possibility that some chromatin-pore complex interaction may be essential.

The object of this communication is to demonstrate that the nuclear pore complex retains its structure (as does the whole nucleus) in the absence, based on both morphological and biochemical grounds, of any membrane.

MATERIALS AND METHODS

Isolation of Nuclei and Nuclear Fractions

Nuclei from rat liver homogenates were prepared as previously described (25, 27) except that the procedure was adapted to larger amounts of tissue. To this intent a crude nuclear fraction was prepared by centrifuging in a previously described (25, 27) except that the procedure interaction may be essential.

swinging bucket rotor. This resulted in a gently packed 2.3 M sucrose-TKM (50 mM Tris-HCl, pH 7.5, at 20°C, 25 mM KCl, 5 mM MgCl2) for 10 min at 600 g in a swinging bucket rotor. This resulted in a gently packed layer of about 12 ml above the cushion and in a supernate. The latter was decanted carefully, avoiding loss of material from the packed layer. This layer was mixed with the cushion by vortexing, and 20 ml of 2.3 M sucrose-TKM was added to give a final concentration of 1.6 M sucrose-TKM. Homogeneous suspension was accomplished by inverting the tube several times. The suspension was transferred to a tube fitting the IEC SB110 rotor (International Electric Centrifuge Co., Needham, Mass.) and underlaid with a cushion of 5 ml 2.3 M sucrose-TKM. Centrifugation for 1 h at 4°C and 25,000 rpm yielded a white pellet of crude nuclei. The nuclei were washed once (25) and resuspended in 0.25 M sucrose-TKM at an optical density of 40.0 A260 per ml. Nuclei were washed once and resuspended in 0.25 M sucrose-TKM to an optical density of 40.0 A260 per ml. Nuclei were counted using a hemacytometer and a phase microscope at × 400 magnification. It was found that 1.0 A260 contained 3.0 ± 0.2 × 10⁶ nuclei. Only freshly prepared, washed nuclei were used for all subsequent experiments.

To nuclear suspensions in 0.25 M sucrose-TKM at an optical density of 25 A260 per ml a solution of 10% vol/vol Triton X-100 (Packard Instrument Co., Inc., Downers Grove, Ill.) was added to give a final concentration of 2% Triton X-100. Controls received equivalent amounts of water. After incubation at 0°C for 10 min, the treated and control samples were underlaid by .5 vol of 1.3 M sucrose-TKM and centrifuged in conical tubes in a swinging bucket rotor for 20 min at 10⁵ g. The supernates were withdrawn and treated at 0°C with 2% Triton X-100. Controls received equivalent amounts of water. After incubation at 0°C for 10 min, the treated and control samples were underlaid by .5 vol of 1.3 M sucrose-TKM and centrifuged in conical tubes in a swinging bucket rotor for 20 min at 10⁵ g. The supernates were withdrawn and treated at 0°C with 2% Triton X-100. Controls received equivalent amounts of water. After incubation at 0°C for 10 min, the treated and control samples were underlaid by .5 vol of 1.3 M sucrose-TKM and centrifuged in conical tubes in a swinging bucket rotor for 20 min at 10⁵ g. The supernates were withdrawn and treated at 0°C with 2% Triton X-100. Controls received equivalent amounts of water. After incubation at 0°C for 10 min, the treated and control samples were underlaid by .5 vol of 1.3 M sucrose-TKM and centrifuged in conical tubes in a swinging bucket rotor for 20 min at 10⁵ g.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Proteins in alcoholic precipitates (67% ethanol at −20°C for 16 h) were suspended in 30% sucrose, 0.1 M Tris-HCl, pH 7.0, by sonication, reduced with 20 mM β-mercaptoethanol in the presence of 4% SDS, and alkylated with 100 mM iodoacetamide. The reduced alkylated proteins were electrophoresed in SDS-polyacrylamide gels by the procedure described by Maizel (32) using a 1-mm thick gel slab apparatus and a 10–15% acrylamide resolving gel.

After electrophoresis the slabs were stained with 0.2% Coomassie blue in 30% methanol, 7% acetic acid and destained In 20% methanol, 7% acetic acid.

Electron Microscopy

Triton-treated or control nuclei were suspended in 0.25 M sucrose, 50 mM triethanolamine-HCl, pH 7.5, 25 mM KCl, 5 mM MgCl2, and sufficient 8% glacial acetic acid (Electron Microscopy Sciences, Fort Washington, Pa.) was added to give a final concentration of 2%. After fixation for 1 h at 0°C the suspension was centrifuged briefly (1,000 g for 5 min). The pellets were resuspended in the same buffer, and a 4% solution of OsO4 (Polysciences, Rydall, Pa.) was added to a final concentration of 1%, and postfixation at 0°C was continued for an additional 3 h. The nuclei were again collected by centrifugation and stained with 0.5% uranyl acetate in acetate-veronal buffer before dehydration and Epon embedding (33, 34).

Sections were cut on a Porter-Blum MT2-B Ultramicrotome (Ivan Sorvall, Inc., Norwalk, Conn.) equipped with a diamond knife (Dupont Instruments, Wilmington, Del.). They were stained with uranyl acetate (35) or treated, as above, with acid for chemical determinations.

Chemical Determinations

Lipids were extracted from the TCA precipitates (28), and phospholipid phosphorus was determined according to Ames (29).

DNA in the PCA precipitates was determined by the method of Burton (30) using calf thymus DNA (Sigma Chemical Co., St. Louis, Mo., Type V, sodium salt) as a standard.

Protein in the PCA precipitates was determined according to Lowry et al. (31). In the case of supernates containing Triton X-100 it was necessary to employ TCA for quantitative precipitation of protein which was then determined after extraction of the pellet with chloroform-methanol to remove the interfering Triton X-100 and TCA.

Robert Peter Aaronson and Günter Blobel

Attachment of the Nuclear Pore Complex 747
RESULTS

Morphology of Control and Triton X-100 Treated Nuclei

Previous work has already established that the structural features recognized in nuclei in situ are retained in nuclei isolated by aqueous sucrose methods (25, 37, 38) (see Fig. 1 a). Furthermore, many reports indicate that gross morphology is unaltered, except for removal of the outer nuclear membrane during isolation of nuclei by detergent solubilization of cytomembranes (27, 39-48) (see Fig. 1 b). Many of these reports claim that the inner nuclear membrane remains essentially intact. Careful examination (compare Figs. 2 and 3) revealed, however, that neither membrane was present. The condensed peripheral chromatin (heterochromatin), the cylindrical channels through the condensed chromatin, and the nuclear pore complexes situated at the peripheral openings of these channels, are apparently unperturbed. It should be noted that in sections normal to the periphery, i.e. cut along the axis of the channel, a nuclear pore complex is observed at the entrance to every cylindrical channel.

The pore complexes appear somewhat more distinct after detergent treatment, presumably owing to removal of the obscuring membranes. In frontal views (Fig. 3 c, d) one sees eight electron-lucent subunits arranged in circular symmetry about a dark central granule (3, 9). In lateral views, pore complexes with two lobes on the outer surface are often observed (Fig. 3 a, c) and occasionally even three lobes are seen (Fig. 3 a, inset).

Chemical Composition of Control and Triton-Treated Nuclei

PHOSPHOLIPIDS: In view of the disparity between our observations and previous reports in terms of the presence or absence of a membrane on detergent-treated nuclei, it was important to determine, in an independent fashion, the presence or absence of some nonspecific membrane marker such as total phospholipid. The data of Table I indicate that, under the given conditions, i.e. at a high concentration of Triton X-100 relative to phospholipid (250:1 wt/wt), 98% of the phospholipid is removed, although essentially all of the DNA remains. These data are, then, consistent with the morphological absence of membranes.

It has been reported (49, 50), however, that detergent treatment may effect only a partial release of nuclear phospholipid, suggestive of a preferential solubilization of the outer nuclear membrane. Thus it was of interest to determine the dependence on detergent concentration of the removal of phospholipid from nuclei. As can be seen in Fig. 4, even relatively low concentrations of Triton X-100 effectively removed almost all the nuclear phospholipid. Finally, it ought to be noted
FIGURE 2 The periphery of isolated rat liver nuclei showing the outer nuclear membrane, *om*; ribosomes, *r*; inner nuclear membrane, *im*; heterochromatin, *h*; and nuclear pore complexes in lateral, *p1*, as well as frontal views, *p2*. The intrachromatin channels leading to the nuclear pores are indicated by arrows. (a) and (b) views normal to the nuclear periphery, × 40,000 and × 120,000, respectively. (c) and (d) views tangential to the nuclear periphery, × 40,000 and × 120,000, respectively. Bars denote 0.1 μm.

that no plateau region, suggestive of a differential response of two classes of phospholipids (two membranes), is evident.

**PROTEIN SPECIES:** As can be seen in Table 1, Triton removed approximately 10% more protein than in the control. In an attempt to determine if this was specific removal, the various nuclear fractions were subjected to SDS-gel electrophoretic analysis. The gels did not fully resolve the multitude of protein species present in the nucleus (Fig. 5, slot 4). The histones (by comparison with authentic histones—not shown) appeared as the most heavily stained bands in the low molecular weight region. The species solubilized by Triton probably represent the major polypeptides of the inner and outer nuclear membranes. A prominent cluster of bands at approximately 52,000 daltons, as well as a single band, normally overshadowed by the histones, at approximately 17,000 daltons, can be recognized (slot 2). It should be noted that no histones are released by the Triton. Since the nuclear pore complex remained attached to the Triton-treated nucleus, it is quite likely that none of the bands solubilized by the detergent represented a significant protein of the complex.

**DISCUSSION**

Intact nuclei have been isolated from a variety of tissues using a number of detergents. Many reports
FIGURE 3 The periphery of isolated rat liver nuclei after treatment with 2% Triton X-100. (a) and (b) views normal to the nuclear periphery, \( \times 40,000 \) and \( \times 120,000 \), respectively. The inset in (a) demonstrates a nuclear pore complex exhibiting three exterior lobes, \( p_3 \), \( \times 40,000 \). (c) and (d) views tangential to the nuclear periphery demonstrating the eightfold rotational symmetry of the nuclear pore complex, \( \times 40,000 \) and \( \times 120,000 \), respectively. Bars denote 0.1 \( \mu \text{m} \).

have claimed, primarily on morphological grounds, that only the outer nuclear membrane was removed. In these cases either different fixation schedules or nuclei from sources other than rat liver presented images of homogeneously granular nuclei surrounded by an amorphous electron-opaque peripheral layer. We have been able to reproduce such images after Triton treatment of
TABLE 1

|                      | Phospholipid | Protein | DNA     |
|----------------------|--------------|---------|---------|
|                      | µg           | µg      | µg      |
| Control              |              |         |         |
| Supernate            | 20 ± 1       | 180 ± 9 | 8 ± 1   |
| Pellet               | 116 ± 5      | 2,900 ± 400 | 1,344 ± 66 |
| Total                | (136)*       | (3,080)* | (1,352)* |
| + Triton X-100       |              |         |         |
| Supernate            | 146 ± 8      | 500 ± 35 | —       |
| Pellet               | 2 ± 1        | 2,700 ± 220 | 1,425 ± 41 |
| Total                | 133          | (3,200)* | (1,425)* |

* Sum of supernate and pellet values.

FIGURE 4 Effect of the concentration of Triton X-100 on removal of nuclear phospholipid. Nuclei at 5 A₂₆₀/µl were incubated and underlaid as in Materials and Methods. After centrifugation and separation of the supernates and pellets 100 µg of bovine plasma albumin were added as a coprecipitant before precipitation with TCA.

FIGURE 5 Electropherogram of a sodium dodecyl sulfate polyacrylamide gel of proteins from treatment of nuclei with Triton X-100. Slot 1, supernate of detergent-treated nuclei; slot 2, supernate of control nuclei. Slot 3, nuclear pellet after detergent treatment. Slot 4, control nuclear pellet. Slot S, molecular weight standards: A, bovine plasma albumin (67,000 daltons); O, ovalbumin (45,000 daltons); C, chymotrypsinogen (23,000 daltons); and H, rabbit hemoglobin (16,000 daltons). Arrows point to the histone bands.

isolated rat liver nuclei (unpublished observation). The presence of this amorphous layer led to the inference that the inner nuclear membrane remained. The absence of phospholipid makes this interpretation unlikely. This layer may be related, as suggested by others (48), to the dense lamella (51), fibrous lamina (52), or zonula nuclium limitans (53).

Phospholipids represent 80-90% of the lipid found in isolated nuclear envelope fractions (19, 21, 53) and are therefore a good marker for the presence of nuclear membranes. Phospholipid determinations subsequent to detergent treatment of nuclei have been published. An undisclosed concentration of Triton X-100 removed approximately 40% of the phospholipid from nuclei obtained from rat kidney cells in culture (49). Treatment of an unreported amount of isolated rat liver nuclei with 1% Triton X-100 released 80% of the phospholipid (50). It is to be expected that composition of buffer, temperature, composition of the membrane, ratio of detergent to phospholipid, length of incubation, type of detergent, and concentration may be critical in determining the release of phospholipid. Indeed, the data of Fig. 4 indicate that the release of phospholipid is a sensitive function of Triton concentration. Furthermore, there is no obvious concentration range in which only 50% of the phospholipid is released, as might be expected if the inner and outer membranes were solubilized differentially. Moreover, if such gross behavior were noted, only...
careful examination in the electron microscope could distinguish between removal of both membranes from half the nuclei and true specific removal of the outer membrane.

Previous reports have also either failed to observe nuclear pores and nuclear pore complexes or have claimed that they were absent from detergent-treated nuclei (27, 39, 40, 43, 44, 48, 54). It is well to note that the nuclear pores should indeed be absent, since the nuclear membranes, which define the pores, are unequivocally absent. The absence of pore complexes from earlier electron micrographs is most likely a function of fixation conditions, since we and others (46, 47) clearly see well-defined structures. In fact, detergent treatment of the nuclei promotes recognition of the pore complexes by removal of the obscuring membranes. The gross ultrastructure of the complex seems undisturbed, although it is possible that the Triton may modify the structure by allowing some loss of material from, or even some nonspecific adherence of material to, the complex.

We would like to emphasize that the nuclear pore complexes do not appear to require the presence of a membrane to retain their structural integrity and their attachment to the periphery of the nucleus. However, we cannot exclude the possibility that a small amount of phospholipid may, in fact, be retained in association with the complex or that the Triton X-100 may itself be bound to the complex replacing the naturally occurring lipid.

Some isolated nuclear envelopes appear to contain pore complexes (19–26), implying that the bulk of the chromatin is also not necessary for structural integrity of the complex. These two lines of evidence, suggesting the nonessential nature of the association of the pore complex with the nuclear membrane and with the chromatin, lend encouragement to our attempts to isolate and characterize structurally intact, morphologically recognizable nuclear pore complexes.

Detergent treatment of isolated nuclei has been performed with the intent of removing (27, 44) and analyzing the ribosomes of the outer nuclear membrane (55, 56). SDS-gel electrophoretic analysis of the low-speed supernate after Triton treatment indicates that ribosomal proteins do not constitute a large class of the proteins solubilized by Triton. Overloading of gels with larger quantities of protein from Triton supernate does, however, show the presence of ribosomal proteins (unpublished observation). However, granules resembling ribosomes are often observed in Triton nuclei (Fig. 3 c), indicating that not all ribosomes may be released by such treatment. Their presence may be explained by nonspecific adsorption, since such granules are observed in the interior as well as on the newly exposed surface of the detergent-treated nuclei. It is not known how much of the original ribosomal population the absorbed granules represent.

We would also like to suggest, as an explanation for the observation that nuclei remain intact, that the often observed heavily staining amorphous layer may be a form of the dense lamella, etc. (51–53). Furthermore, we propose that this layer is responsible for the organization of the nuclear material into a relatively compact mass and that the nuclear pore complexes may be attached to this layer rather than to the chromatin.

We wish to thank Dr. George E. Palade for his helpful advice during the course of this work.

This work was supported by United States Public Health Service Postdoctoral Fellowship (Robert Peter Aaronson) number 1 F02 GM53297-01 and by grant 5 RO1 Ca 12413-02 from the National Cancer Institute.

Portions of this manuscript were presented at the Thirteenth Annual Meeting of the American Society for Cell Biology, Miami Beach, Fla., November, 1973, J. Cell Biol. 59 (2, Pt. 2): a.

Received for publication 14 March 1974.

REFERENCES

1. WATSON, M. L. 1954. Pores in the mammalian nuclear membrane. Biochim. Biophys. Acta. 15:475–479.
2. WATSON, M. L. 1955. The nuclear envelope: its structure and relation to cytoplasmic membranes. J. Biochem. Biophys. Cytol. 1:257–270.
3. STEVENS, B. J., and J. ANDRÉ. 1969. The nuclear envelope. In Handbook of Molecular Cytology. A. Lima-de-Faria, editor. Interscience Pubs., Inc., John Wiley and Sons, Inc., New York. 837–871.
4. FRANKE, W. W., and U. SCHEER. 1970. The ultrastructure of the nuclear envelope of amphibian oocytes: a reinvestigation. 1. The mature oocyte. J. Ultrastruct. Res. 30:288–316.
5. FRANKE, W. W. 1970. Universality of the nuclear pore complex structure. Z. Zellforsch. Mikrosk. Anat. 105:405–429.
6. KARTENBECK, J., H. ZENTGRAF, U. SCHEER, and W. W. FRANKE. 1971. The nuclear envelope in freeze-etching. Advan. Anat. Embryol. Cell Biol. 45:1–55.
7. KESSEL, R. G. 1973. Structure and function of the nuclear envelope and related cytomembranes. In Progress in Surface and Membrane Science. J. F. Danielli, M. D. Rosenberg, and D. H. Codinhead, editors. Academic Press, Inc., New York. 6:243–329.

8. KAY, R. R., and I. R. JOHNSTON. 1973. The nuclear envelope: current problems of structure and of function. Sub-Cell. Biochem. 2:127–166.

9. FELDHERR, C. M. 1972. Structure and function of the nuclear envelope. II. Ultrastructure. In Advances in Cell and Molecular Biology. E. J. DuPraw, editor. Academic Press, Inc., New York. 2:273–307.

10. STEVENS, B. J., and H. SWIFT. 1966. RNA transport from nucleus to cytoplasm in Chironimus salivary glands. J. Cell Biol. 31:55–77.

11. FRANKE, W. W. 1970. Nuclear pore flow rate. Naturwissenschaften. 57:44–45.

12. SIEBERT, G. 1972. The biochemical environment of the mammalian nucleus. Sub-Cell. Biochem. 1:277–292.

13. DU PRAW, E. J. 1965. Macromolecular organization of nuclei and chromosomes: a folded-fibre model based on whole mount electron microscopy. Nature (Lond.). 206:338–343.

14. BEAMS, H. W., and SHIRLEY MUELLER. 1970. Effects of ultracentrifugation on the interphase nucleus of somatic cells with special reference to the nuclear envelope-chromatin relationships. Z. Zellforsch. Mikros. Anat. 108:297–308.

15. COMINGS, D. E., and T. A. OKADA. 1970. Association of chromatin fibers with the annuli of the nuclear membrane. Exp. Cell Res. 62:293–302.

16. ENGELHARDT, P., and K. PUSA. 1972. Nuclear pore complexes: "Press-Stud" elements of chromosomes in pairing and control. Nat. New Biol. 240:163–166.

17. FRANKE, W. W., and H. FALK. 1970. Appearance of nuclear pore complexes after Bernhardt's staining procedure. Histochemie. 24:266–278.

18. SCHEER, U. 1972. The ultrastructure of the nuclear envelope of amphibian oocytes. IV. On the chemical nature of the nuclear pore material. Z. Zellforsch. Mikros. Anat. 127:127–148.

19. KASHING, D. M., and C. B. KASPER. 1969. Isolation, morphology, and composition of the nuclear membrane from rat liver. J. Biol. Chem. 244:3,786–3,792.

20. BEREZNEY, R., L. K. FUNK, and F. L. CRANE. 1970. The isolation of nuclear membrane from a large-scale preparation of bovine liver nuclei. Biochim. Biophys. Acta. 203:531–546.

21. FRANKE, W. W., B. DEUMLING, B. ERNEN, E. D. JARASCH, and H. KLEINING. 1970. Nuclear membranes from mammalian liver. I. Isolation procedure and general characterization. J. Cell Biol. 46:379–395.

22. ZENTGRAF, H., B. DEUMLING, E. D. JARASCH, and W. W. FRANKE. 1971. Nuclear membranes and plasma membranes from hen erythrocytes. I. Isolation, characterization, and comparison. J. Biol. Chem. 246:2,986–2,995.

23. AGUTTER, P. S. 1972. The isolation of the envelopes of rat liver nuclei. Biochim. Biophys. Acta. 255:397–401.

24. KAY, R. R., D. FRASER, and I. R. JOHNSTON. 1972. A method for the rapid isolation of nuclear membranes from rat liver. Eur. J. Biochem. 30:145–154.

25. MÖNNERON, A., G. BLOBEL, and G. E. PALADE. 1972. Fractionation of the nucleus by divalent cations. Isolation of nuclear membranes. J. Cell Biol. 55:104–125.

26. BORNENS, M. 1973. Action of heparin on nuclei: solubilization of chromatin enabling the isolation of nuclear membranes. Nature (Lond.). 244:28–30.

27. BLOBEL, G., and V. R. POTTER. 1966. Nuclei from rat liver: isolation method that combines purity with high yield. Science (Wash. D. C.). 154:1,662–1,665.

28. FOLCH, J., M. LEES, and G. H. SLOANE STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

29. AMES, B. N. 1967. Assay of inorganic phosphate, total phosphate, and phosphatase. In Methods in Enzymology. E. Neufeld and V. Ginsburg, editors. Academic Press, Inc., New York. 8:115–118.

30. BURTON, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemistry. 62:315–323.

31. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265–275.

32. MAIZEL, J. V. 1969. Acrylamide gel electrophoresis of proteins and nucleic acids. In Fundamental Techniques in Virology. K. Habel and N. P. Saltman, editors. Academic Press, Inc., New York. 334–362.

33. LUFT, G. H. 1961. Improvements in epoxy embedding methods. J. Biophys. Biochem. Cytol. 9:409–414.

34. FARQUHAR, M. G., and G. E. PALADE. 1965. Cell junctions in amphibian skin. J. Cell Biol. 26:263–291.

35. WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol. 4:475–478.

36. VENABLE, J. H., and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. 25:407–408.

37. DAVISON, P. F., and E. H. MEYER. 1956. Electron microscopy of cell nuclei isolated in aqueous media. Exp. Cell Res. 11:237–239.

38. MAGGIO, R., P. SIEKEVITZ, and G. E. PALADE. 1963. Studies on isolated nuclei. I. Isolation and chemical characterization of a nuclear fraction from guinea pig liver. J. Cell Biol. 18:267–291.
39. Fisher, H. W., and H. Harris. 1962. The isolation of nuclei from animal cells in culture. Proc. R. Soc. Lond. B Biol. Sci. 156:521–523.
40. Hubert, M.-T., P. Favard, N. Carasso, R. Rozencwajg, and J.-P. Zalta. 1962. Méthode d’isolement de noyaux cellulaires à partir de foie de rat. J. Microsc. (Paris). 1:435–444.
41. Hymer, W. C., and E. L. Kuff. 1964. Isolation of nuclei from mammalian tissues through the use of Triton X-100. J. Histochem. Cytochem. 12:359–363.
42. O’Brien, B. R. A. 1964. A rapid method for the isolation and collection of nuclei from whole cell suspensions. J. Cell Biol. 20:525–528.
43. Hadjiolov, A. A., Z. S. Tencheva, and A. G. Bojadjieva-Mikhailova. 1965. Isolation and some characteristics of cell nuclei from brain cortex of adult cat. J. Cell Biol. 26:383–393.
44. Holtzman, E., I. Smith, and S. Penman. 1966. Electron microscopic studies of detergent-treated HeLa cell nuclei. J. Mol. Biol. 17:131–135.
45. Lovtrup-Rein, H., and B. S. McEwen. 1966. Isolation and fractionation of rat brain nuclei. J. Cell Biol. 30:405–415.
46. Sadowski, P. D., and J. W. Steiner. 1968. Electron microscopic and biochemical characteristics of nuclei and nucleoli isolated from rat liver. J. Cell Biol. 37:147–161.
47. Chardonnnet, Y., and S. Dales. 1972. Early events in the interaction of adenoviruses with HeLa cells. III. Relationships between an ATPase activity in nuclear envelopes and transfer of core material: a hypothesis. Virology. 48:342–359.
48. Laval, M., and M. Bouteille. 1973. Synthetic activity of isolated rat-liver nuclei. I. Ultrastructural study at various steps of isolation. Exp. Cell. Res. 76:337–348.
49. Ben-Porat, T., and A. S. Kaplan. 1971. Phospholipid metabolism of herpesvirus-infected and uninfected rabbit kidney cells. Virology. 45:252–264.
50. Tata, J. R., M. J. Hamilton, and R. D. Cole. 1972. Membrane phospholipids associated with nuclei and chromatin: melting profile, template activity, and stability of chromatin. J. Mol. Biol. 67:231–246.
51. Kalifat, S. R., M. Bouteille, and J. J. Delarme. 1967. Etude structurale de la lamelle dense observée au contact de la membrane nucléaire interne. J. Microsc. (Paris). 61:1019–1026.
52. Fawcett, D. W. 1966. On the occurrence of a fibrous lamina on the inner aspect of the nuclear envelope in certain cells of vertebrates. Am. J. Anat. 119:129–146.
53. Patrizi, G., and M. Poger. 1967. The ultrastructure of the nuclear periphery. J. Ultrastruct. Res. 17:127–136.
54. Zalta, J.-P., R. Rozencwajg, N. Carasso, and P. Favard. 1962. Isolement d’une fraction de noyaux cellulaires dont la pureté est contrôlée au microscope électronique. C. R. Hebld. Seances. Acad. Sci. 255:412–414.
55. Whittle, E. D., D. E. Bushnell, and V. R. Potter. 1968. RNA associated with the outer membrane of rat liver nuclei. Biochim. Biophys. Acta. 161:41–50.
56. Sadowski, P. D., and J. A. Howden. 1968. Isolation of two distinct classes of polysomes from a nuclear fraction of rat liver. J. Cell Biol. 37:163–181.