Generation of an Internal Matrix in Mature Avian Erythrocyte Nuclei during Reactivation in Cytoplasts

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ABSTRACT When fused with mouse L-cell cytoplasts, chick erythrocyte nuclei enlarge, take up proteins from the host cytoplasm, and recommence RNA synthesis. We found that during this transition the erythrocyte nuclei gain an internal nuclear matrix, thus providing a novel approach to questions concerning the nature of the salt-resistant intranuclear skeleton. A new method for preparation and examination of the nuclear matrix in situ is also described.

The nuclear matrix is defined as the residual structure which remains after nuclei are sequentially subjected to DNase, low salt, and high salt treatments; additional extractions with nucleases and non-ionic detergents may also be included (reviewed in 4, 44). Despite the loss of ~90% of nuclear protein and up to 99% of nucleic acids, the final matrix retains the approximate shape and internal organization of the original nucleus. Three structural domains of matrix have been recognized: the peripheral pore complex-lamina, the residual nucleolus, and the interchromatinic or internal matrix (4, 44). The pore-lamina has been extensively characterized: its major components are a family of related structural proteins, the lamins (17, 18, 26, 27, 44, 45), which seem to be confined to the nuclear perimeter (17, 18, 26). The internal matrix, however, contains a large number of polypeptides, most of which have yet to be studied (22, 40, 47).

Current interest in the nuclear matrix has been stimulated by reports that the matrix is specifically associated with a number of key nuclear components, including newly synthesized DNA (6, 7, 9, 14, 23, 38), newly synthesized RNA (2, 16, 20, 21, 24, 33, 34, 36, 47), steroid hormone receptors (2, 3), specific genes and families of repetitious DNA (13, 37, 39, 42, 46), and the polyoma T antigen (9). Recently, additional evidence has accumulated to implicate the matrix in RNA processing (12, 20, 35, 43). On the basis of these results, it has been hypothesized that the internal nuclear matrix provides a skeletal framework in vivo upon and around which chromatin is organized and transcription and replication facilitated.

We showed recently that inactive chick erythrocyte nuclei lack an internal nuclear matrix (29, 31). The fact that these nuclei can be "reactivated" with respect to chromatin condensation and recovery of RNA and DNA synthesis if they are fused with another cell or cytoplast (1, 15, 19, 28, 32) suggested that it would be of interest to examine the status of the nuclear matrix during the reactivation process. We find that within 16 h of fusion with mouse L-cell cytoplasts the chick erythrocyte nucleus gains an internal nuclear matrix. A preliminary account of this work has already been published (30). As discussed below, this system opens the way to definitive tests of various aspects of the nuclear matrix hypothesis.

MATERIALS AND METHODS

Cell Cultures: Mouse L-929 cells were maintained in minimal essential medium (Eagle) supplemented with 5% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. For cell fusion experiments, cells were released from the flasks with trypsin and plated on 60-mm culture dishes so that individual cells were not touching each other. After a 12-h incubation, enucleation was carried out.

Enucleation and Cell Fusion: These procedures were based on those described by Lipsich et al. (32). The cells were treated with 10 μg/ml cytochalasin B (Aldrich Chemical Co., Inc., Milwaukee, WI) in growth medium for 20 min, following which the nuclei were released by centrifuging the inverted culture dishes at 12,000 *g* for 45 min at 37°C (GSA rotor, Sorvall RC-5 centrifuge; DuPont Instruments–Sorvall Biomedical Div., DuPont Co., Wilmington, DE). To achieve this, the dishes were inverted in 150 ml of medium containing 10 μg/ml cytochalasin B in flat-bottomed centrifuge bottles from which the tops had been cut. After centrifugation, the dishes were removed and the cytoplasts were given fresh medium and allowed to recover for 90 min. One dish was then stained with Giemsa's to monitor the enucleation efficiency. Sendai virus, inactivated by UV irradiation, was then added at a concentration of 200 haemagglutination U/ml in Earle's balanced salt solution at 4°C. After 5 min, excess virus was removed and the cells were overlaid with a suspension of 14–18-d chick embryo erythrocytes (about 10^8 per dish) in balanced salt solution. The dishes were then incubated at 4°C for 15 min and then transferred to a 37°C incubator. Following a further 60-min incubation, the unfused erythrocytes were washed off and the reconstituted cells supplied with fresh complete medium. For most of the experiments reported here, the cells were examined after 16-h of incubation.

Nuclear Matrix Production In Situ: Cell monolayers on 60-mm culture dishes were first overlaid with collagen (11). The stock solution of 0.2% collagen (Type VII, Sigma Chemical Co., St. Louis, MO) in 0.1% acetic acid was neutralized and diluted into minimal essential medium to a final concentration of 0.16% just before use. After removing the culture medium, 0.4 ml of ice-cold collagen suspension was placed in each dish, and the cells were incubated at 37°C for 10–15 min. During the incubation, collagen monomers polymerize to form a

protective skin over the cells, which can then be irrigated with aqueous solutions without becoming detached from the plastic. To render the cells permeable to DNase, they were first treated with 0.1% Triton X-100 containing 0.001% spermine for 15 min at 0°C. Other detergent mixtures tried were 0.1% Triton X-100, 5 mM $MgCl_2$, 25 mM KCl, 10 mM Tris; pH 7.5, and 0.1% Nonidet P-40, 5 mM $MgCl_2$, 130 mM KCl, 15 mM NaCl, 10 mM PIPES, pH 7.5. These attempts to devise a more "physiologically balanced" permeabilizing medium had little effect on the appearance of L-cell nuclei or nuclear matrix. After detergent treatment, the cells were washed extensively with DNase buffer (5 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Tris, pH 7.4) and then digested with 200 μg/ml DNase I (Worthington Biomedical Corp., Freehold, NJ) for 30 min at 0°C in the same buffer. A further wash with digestion buffer or 0.2 mM $MgCl_2$, 10 mM Tris, pH 7.4, was followed by extraction with 2.0 M NaCl, 0.2 mM $MgCl_2$, 10 mM Tris, pH 7.4 (44), for 90 min at 0°C. Finally, the cells were washed once more with low salt buffer before preparation for electron microscopy. The protease inhibitor, phenylmethylsulfonyl fluoride, was included at a concentration of 1.0 mM in the detergent and high salt solutions, and in some cases 1 mM sodium tetrathionate was also included (5). To test for the efficiency of the DNase treatment, some plates were washed with PBS (0.14 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2), then stained for 15 min with acridine orange, ethidium bromide, or Hoechst 33258, all at concentrations of 10 μg/ml in PBS. After further thorough washing with PBS, the dishes were drained, a drop of 50% glycerol in PBS was added, and a coverslip placed over the cells. DNA fluorescence was measured using a Leitz microscope with epi-illumination and quantitative fluorescence attachments. For each treatment, the fluorescence from 20 to 40 individual nuclei was measured and corrected for background.

Electron Microscopy: Cells at each stage in the experiment were fixed on the culture dishes for electron microscopy. After rinsing with 0.1 M sodium phosphate buffer pH 7.4 containing 2.5% sucrose, the cells were fixed for 30 min at room temperature with 2% glutaraldehyde in the same buffer, washed three times with buffer, and postfixed with 1% osmium tetroxide in phosphate buffer. Following a brief water wash, the cultures were stained with 2% aqueous uranyl acetate, washed again, and then stained briefly with aqueous toluidine blue. This latter procedure greatly facilitated the selection of areas for examination and did not affect the ultrastructure of the cells. Finally, after dehydration in an ethanol series, the cells were embedded in an Epon-Araldite mixture. Once the resin had set, it was possible to release the cells from the plastic dish for sectioning parallel to the plane of the cell monolayer. Sections were cut on a MT-2 ultramicrotome (DuPont-Sorvall), stained with lead citrate and uranyl acetate, and examined in a Siemens 102A electron microscope operated at 60 kV.

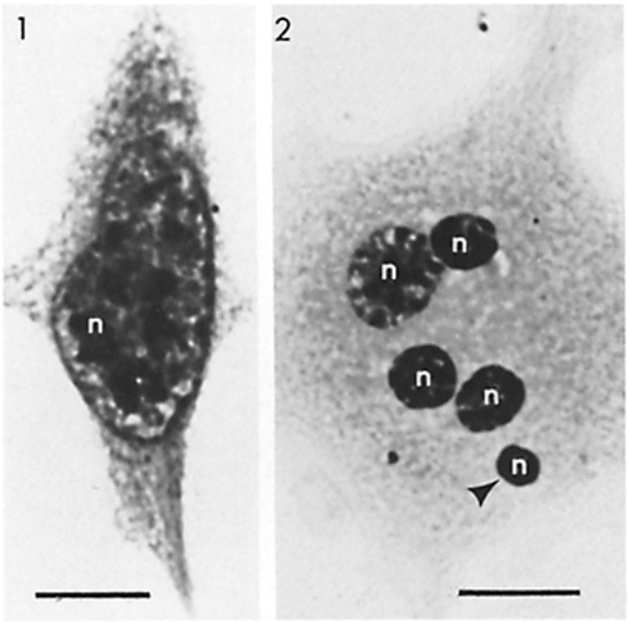
RESULTS

Using the cytochalasin B technique (32, 41), it was possible to obtain >99% enucleation of mouse L-cells (Table I). Counts of

TABLE I
Counts of Enucleation and Fusion Efficiency after 16-h Reactivation

	% of cells
L-cell nucleus retained (not enucleated)	0.5
Without nuclei, or with one or more erythrocyte nuclei	99.5
Of cells with erythrocyte nuclei, number of nuclei per reconstituted cell	
1	64
2	27
3	8
4	1
5	1

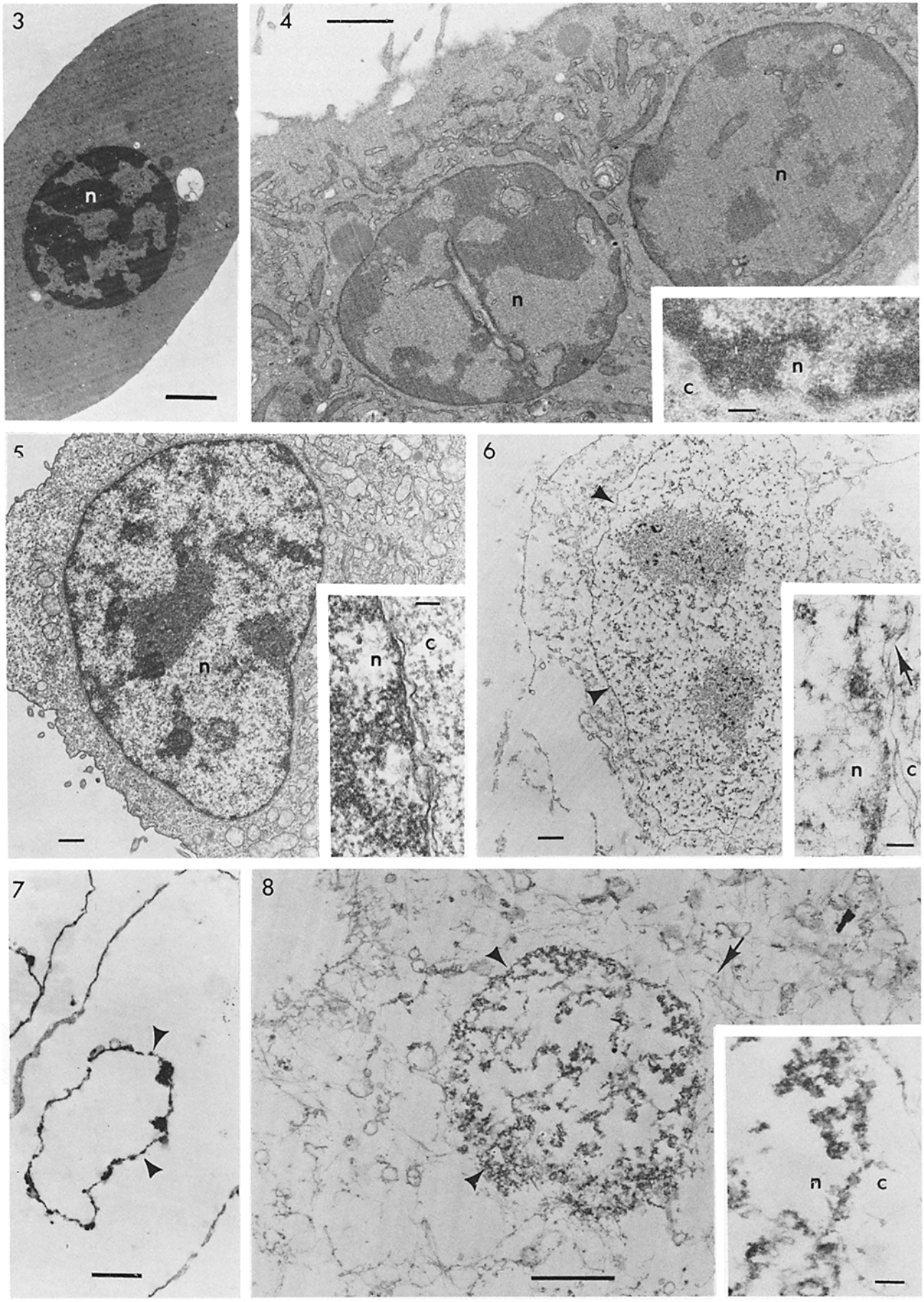
Data represent the average of two experiments; total $n = 900$ cells.



FIGURES 1 and 2 Giemsa's stain of fusions between L-cell cytoplasts and 17-d chick erythrocytes after 16-h incubation. Fig. 1 is an example of the 0.5% of L-cells which fail to become enucleated. Fig. 2 shows a cytoplast containing four reactivating chick nuclei. A fifth nucleus (arrowhead) lies on top of the cytoplast and indicates the size of the unreactivated erythrocyte nucleus. (*n*) Nuclei. Bar. 10 μm. × 1,500.

Giemsa-stained plates (Figs. 1 and 2) after fusion indicated that 55% of cytoplasts had taken up at least one erythrocyte nucleus, some of them more than one (Fig. 2, Table I). The implanted nuclei showed different degrees of reactivation as judged by their size, which increased up to a maximum tenfold volume change (assuming that the nuclei remained spherical). Electron micrographs of the reactivated nuclei showed a dramatic decondensation of the chromatin when compared with the starting material (Figs. 3 and 4), and granular material appeared in the interchromatinic areas. To study the nuclear matrix in reconstituted cells, we devised an in situ method of revealing the matrix. This provides two important advantages: reactivated nuclei do not have to be isolated, and there is no possibility that matrices derived from contaminating L-cell nuclei will be mistaken for erythrocyte material. The in situ technique is analogous to matrix preparation in isolated nuclei, the detergent-permeabilized cells being treated sequentially with DNase, low magnesium buffer (0.2 mM $MgCl_2$, 10 mM Tris, pH 7.4), and high salt buffer (0.2 mM $MgCl_2$, 2.0 M NaCl, 10 mM Tris, pH 7.4; see Materials and Methods for details). To determine whether the DNase treatment was effective under these circumstances, nuclear DNA content was measured before and after digestion using the DNA fluoro-

FIGURES 3–8 Electron micrographs of untreated cells and nuclear matrix preparations using the collagen overlay method. Bars (Figs. 3–8) 1 μm; (*insets*) 0.1 μm. (*n*) Nucleus. (*c*) Cytoplasm. Fig. 3: Untreated 17-d embryonic erythrocyte. Fig. 4: Cytoplast containing two reactivating erythrocyte nuclei 16 h postfusion. Extensive chromatin decondensation has occurred. *Inset* shows nucleus-cytoplasm boundary from another preparation. Fig. 5: Untreated L-cell with *inset* showing nucleus-cytoplasm boundary. Fig. 6: L-cell after in situ matrix production. The boundary of the nucleus (pore-complex lamina) is indicated by arrowheads and shown enlarged in the *inset*. Arrow on *inset* shows intermediate filaments in the residual cytoplasm. Fig. 7: 17-d embryonic erythrocyte after in situ matrix production. Nuclear material is restricted mainly to the pore-complex lamina (arrowheads). Fig. 8: Reactivating erythrocyte 16 h postfusion after nuclear matrix preparation. In contrast to Fig. 7, an internal matrix is present. Arrowheads indicate nucleus-cytoplasm boundary which is enlarged in the *inset*. Arrow points to intermediate filaments. (Figs. 3 and 7) × 10,000. (Fig. 4) × 12,000. (Figs. 5 and 6) × 5,000. (Fig. 8) × 15,000.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chromes acridine orange, ethidium bromide, and Hoechst 33258. The results showed that 90–95% of nuclear DNA was removed during the 30-min DNase treatment at 0°C. This can be compared with the removal of ~75% of DNA during the initial DNase digestion of isolated nuclei prior to nuclear matrix isolation (4). Subsequent high salt treatment reduced the DNA content (as measured by fluorescence) still further, indicating that the in situ method provides an extraction process analogous to the standard nuclear matrix isolation procedure.

To prevent the cell and cell contents from becoming dislodged from the culture dish, they were first overlaid with a thin layer of collagen which provides a fully permeable protective sheet. After collagen treatment, erythrocytes and L-cells retain their normal ultrastructure (Figs. 3 and 5). When treated by the in situ method, L-cells show a typical nuclear matrix, with pore complex-lamina, nucleolar matrix, and interchromatinic matrix clearly visible (Fig. 6). The salt-resistant cytoskeletal elements, including intermediate filaments, are well-preserved, although the plasma membrane is heavily damaged (Fig. 6). A similar preservation of both nuclear and cytoskeletal elements in critical-point-dried material has recently been reported (10).

As expected from earlier results (29, 31), whole erythrocytes from 14–18-d embryos lack an internal matrix after this treatment (Fig. 7). Some whole erythrocytes remain attached to the outside of the cytoplasts during fusion and provide a useful internal control for the matrix preparation technique. In contrast to the original nuclei (Fig. 7), reactivated nuclei show a distinct internal matrix 16 h postfusion (Fig. 8). In general, the amount of internal matrix material was proportional to the extent of reactivation, as judged by the size increase of the nucleus: the larger the nucleus, the greater the amount of internal matrix present. This is illustrated in Figs. 9–11 which depict matrix preparations of erythrocyte nuclei which, by light microscopic examination (c.f., Fig. 2), showed no increase in size (Fig. 9) or little increase in size (Figs. 10 and 11) after incorporation into L-cell cytoplasts. The paucity of internal matrix material in these cases allows us to reject the possibility that the mere placement of an erythrocyte nucleus in a cytoplast results in the generation of an internal matrix, as would be observed if it were due to a failure of the extraction process.

Sodium tetrathionate has been shown to increase the amount of protein in the nuclear matrix, as well as the proportion of newly replicated DNA associated with the matrix (5), and was included in some experiments to ensure that even under conditions most favorable for matrix formation unreactivated mature erythrocytes really lacked an internal matrix (Figs. 7 and 9–11). However, it has recently been shown that much of the action of tetrathionate can be attributed to its property of promoting S-S bonding (25). This raises the possibility that matrix structures may be induced by the reagent and suggests that it should be used with caution in nuclear matrix experiments. In the procedure described here, sodium tetrathionate did not induce an internal matrix in unreactivated erythrocyte nuclei, nor did it affect the ultrastructure of L-cell nuclear matrices.

DISCUSSION

We have shown that during reactivation of erythrocyte nuclei in mouse L-cell cytoplasts, an internal nuclear matrix is generated. The new matrix presumably arises from proteins taken up from the cytoplasm by the implanted nucleus, indicating that each cytoplast either has or can synthesize sufficient protein to generate a matrix in several erythrocyte nuclei. In heterokaryons, it has been shown that such protein uptake is not random but includes nuclear marker proteins and excludes cytoplasmic marker proteins (1). Lipsich et al. (32) have shown that many of the morphological features of reactivation in cytoplast-erythrocyte hybrids, including nuclear swelling and chromatin decondensation, occur in the presence of cycloheximide. This may suggest that a large proportion of the matrix-forming proteins pre-exist in the cytoplasm and are not synthesized in response to the implanted nucleus.

The generation of an internal matrix under these conditions provides an experimental framework within which some of the properties of the matrix can be tested. For example, it will be possible to determine which polypeptide species contribute to the erythrocyte matrix and to compare them to L-cell matrix polypeptides. The nuclear matrix hypothesis would predict that the two sets of polypeptides should be very similar. Also, a comparison can be made between the erythrocyte matrix proteins accumulated by nuclei reactivated in L-cells and those in nuclei reactivated in chick fibroblast cytoplasts (8). Finally, studies are in progress to relate the time course of matrix generation to the time course of transcriptional recovery and to the onset of DNA synthesis. Correlations between these

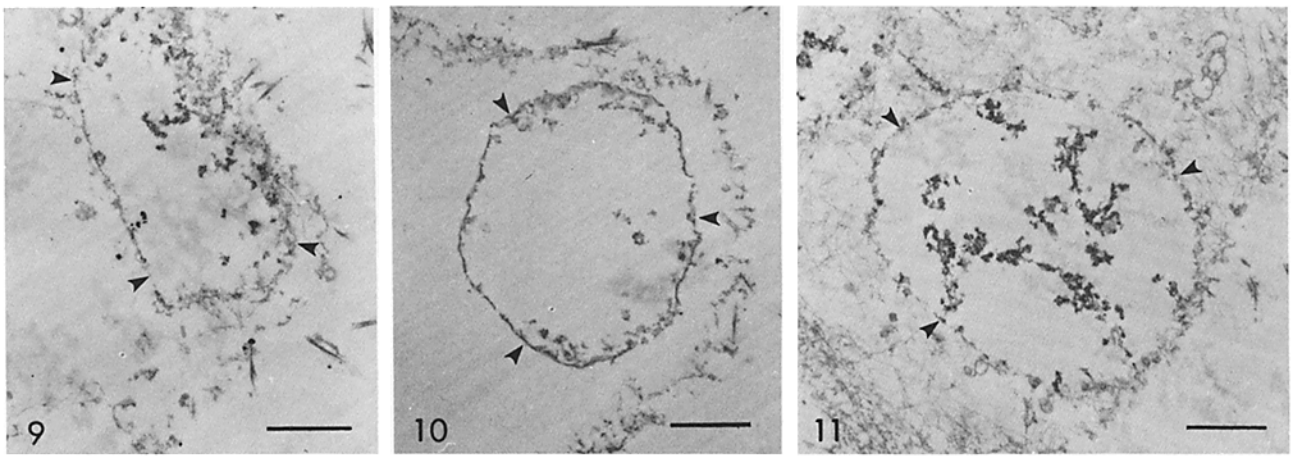

FIGURE 9–11 Erythrocyte nuclei which were implanted in L-cell cytoplasts but showed little or no swelling or other signs of reactivation. In contrast to Fig. 8, little internal matrix material is present. Arrowheads indicate nucleus-cytoplasm boundary. Bars, 1 μm. × 11,000.

nuclear activities and internal matrix generation would support the hypothesis that the matrix plays a functional role in such metabolic events.

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REFERENCES

1. Appels, R., and N. R. Ringertz. 1975. Chemical and structural changes within chick erythrocyte nuclei introduced into mammalian cells by fusion. *Curr. Top. Dev. Biol.* 9:137–166.
2. Agutter, P. S., and K. Birchall. 1979. Functional differences between mammalian nuclear protein matrices and pore-lamina complex laminae. *Exp. Cell Res.* 124:453–460.
3. Barrack, E. R., and D. S Coffey. 1980. The specific binding of estrogens and androgens to the nuclear matrix of sex hormone responsive tissues. *J. Biol. Chem.* 255:7265–7275.
4. Berezney, R. 1979. Dynamic properties of the nuclear matrix. *In* The Cell Nucleus. H. Busch, editor. Academic Press, Inc., New York. 7:413–456.
5. Berezney, R. 1979. Effect of protease inhibitors on matrix proteins and the association of replicating DNA. *Exp. Cell Res.* 123:411–414.
6. Berezney, R., and L. A. Buchholtz. 1981. Dynamic association of replicating DNA fragments with the nuclear matrix of regenerating liver. *Exp. Cell Res.* 132:1–13.
7. Berezney, R., and D. S. Coffey. 1975. Nuclear protein matrix: association with newly synthesized DNA. *Science (Wash. DC).* 189:291–293.
8. Bruno, J., N. Reich, and J. J. Lucas. 1981. Globin synthesis in hybrid cells constructed by transplantation of dormant avian erythrocyte nuclei into enucleated fibroblasts. *Mol. Cell. Biol.* 1:1163–1176.
9. Buckler-White, A. J., G. W. Humphrey, and V. Pigiet. 1980. Association of polyoma T antigen and DNA with the nuclear matrix from lytically infected 3T6 cells. *Cell* 22:37–46.
10. Capco, D. G., K. M. Wan, and S. Penman. 1982. The nuclear matrix: three dimensional architecture and protein composition. *Cell* 29:847–858.
11. Chambard, M., J. Gabrion, and J. Mauchamp. 1981. Influence of collagen gel on the orientation of epithelial cell polarity. *J. Cell Biol.* 91:157–166.
12. Ciejek, E. M., J. L. Nordstrom, M-J Tsai, and B. W. O'Malley. 1982. Ribonucleic acid precursors are associated with chick oviduct nuclear matrix. *Biochemistry.* 21:4945–4953.
13. Cook, P. R., and I.A. Brazell. 1980. Mapping sequences of nuclear DNA by their progressive detachment from the nuclear cage. *Nucleic Acids Res.* 8:2895–2906.
14. Dijkwel, P. A., L. H. F. Mullenders, and F. Wanka. 1979. Analysis of the attachment of replicating DNA to a nuclear matrix in mammalian interphase nuclei. *Nucleic Acids Res.* 6:219–230.
15. Ege, T., J. Zeuthen, and N. R. Ringertz. 1975. Reactivation of chick erythrocyte nuclei after fusion with enucleated cells. *Somatic Cell Genet.* 1:65–80.
16. Faiferman, I., and A. O. Pogo. 1975. Isolation of nuclear ribonucleoprotein network that contains heterogeneous RNA and is bound to the nuclear envelope. *Biochemistry.* 14:3808–3816.
17. Franke, W. W., V. Scheer, G. Krohne, and E-D. Jarasch. 1981. The nuclear envelope and the architecture of the nuclear periphery. *J. Cell Biol.* 91(3, Pt. 2):39s–50s.
18. Gerace, L., A. Blum, and G. Blobel. 1978. Immunocytochemical localization of the major peptides of the nuclear pore-complex lamina fraction. *J. Cell Biol.* 79:546–566.
19. Harris, H. 1970. Nucleus and Cytoplasm. Clarendon Press, Oxford.
20. Herlan, G., W. A. Eckert, W. Kaffenberger, and F. Wunderlich. 1979. Isolation and characterization of an RNA-containing nuclear matrix from *Tetrahymena* macronuclei. *Biochemistry.* 18:1782–1788.
21. Herman, R., L. Weymouth, and S. Penman. 1978. Heterogenous nuclear RNA-protein fibers in chromatin—depleted nuclei. *J. Cell Biol.* 78:663–673.
22. Hodge, L. D., P. Mancini, F. M. Davis, and P. Heywood. 1977. Nuclear matrix of HeLa S_3 cells. *J. Cell Biol.* 72:194–208.
23. Hunt, B. F., and B. Vogelstein. 1982. Association of newly replicated DNA with the nuclear matrix of *Physarum polycephalum*. *Nucleic Acids Res.* 9:349–363.
24. Jackson, D. A., S. J. McCready, and P. R. Cook. 1981. RNA is synthesized at the nuclear cage. *Nature (Lond.).* 292:552–555.
25. Kaufmann, S. H., D. S. Coffey, and H. H. Shaper. 1981. Considerations in the isolation of rat liver nuclear matrix, nuclear envelope, and pore complex lamina. *Exp. Cell Res.* 132:105–123.
26. Krohne, G., W. W. Franke, S. Ely, A. D'Arcy, and E. Jost. 1978. Localization of a nuclear envelope associated protein by indirect immunofluorescence microscopy using antibodies against a major polypeptide from rat liver fractions enriched in nuclear envelope-associated material. *Cytobiologie.* 18:22–38.
27. Krohne, G., W. W. Franke, and V. Scheer. 1978. Major polypeptides of the nuclear pore complex. *Exp. Cell Res.* 116:85–102.
28. Ladda, R. L., and R. D. Estensen. 1970. Introduction of a heterologous nucleus into enucleated cytoplasms of cultured mouse L-cells. *Proc. Natl. Acad. Sci. USA.* 67:1528–1533.
29. LaFond, R. E., and C. L. F. Woodcock. 1981. The status of the nuclear matrix in adult chicken erythrocyte nuclei. *J. Cell Biol.* 91(2, Pt. 2): 57*a*. (Abstr.)
30. LaFond, R. E., C. L. F. Woodcock, E. R. Kundahl, and J. J. Lucas. 1982. Internal components of the nuclear matrix are generated in reactivated chick erythrocyte nuclei. *J. Cell Biol.* 95(2, Pt. 2): 75*a*. (Abstr.)
31. LaFond, R. E., and C. L. F. Woodcock. 1983. Status of the nuclear matrix in mature and embryonic erythrocyte nuclei. *Exp. Cell Res.* In press.
32. Lipsich, L. A., J. J. Lucas, and J. R. Kates. 1978. Cell cycle dependence of the reactivation of chick erythrocyte nuclei after transplantation into mouse L929 cell cytoplasts. *J. Cell Physiol.* 97:199–208.
33. Long, B. H., C.-Y. Huang, and A. O. Pogo. 1979. Isolation and characterization of the nuclear matrix in Friend Erythroleukemia cells: chromatin and HnRNA interactions with the matrix. *Cell* 18:1079–1090.
34. Maundrell, K., E. S. Maxwell, E. Puvion, and K. Scherrer. 1981. The nuclear matrix of chick erythroblasts is associated with globin mRNA coding sequences but not with the major proteins of 40S nuclear RNP. *Exp. Cell Res.* 136:435–445.
35. Mariman, E. C. M., C. A. G. van Eekelen, R. J. Reinders, A. J. M. Berns, and W. van Venrooij. 1982. Adenoviral heterogeneous nuclear RNA is associated with the host nuclear matrix during splicing. *J. Mol. Biol.* 154:103–119.
36. Miller, T. E., C.-Y. Huang, and A. O. Pogo. 1978. Rat liver nuclear skeleton and ribonucleoprotein complexes containing hnRNA. *J. Cell Biol.* 76:675–691.
37. Nelkin, B. D., D.M. Pardoll, and B. Vogelstein. 1980. Localization of SV 40 genes within supercoiled loop domain. *Nucleic Acids Res.* 8:5623–5633.
38. Pardoll, D. M., Vogelstein, B., and Coffey, D. S. 1980. A fixed site of DNA replication in eukaryotic cells. *Cell* 19:527–536.
39. Pardoll, D. M., and B. Vogelstein. 1980. Sequence analysis of nuclear matrix associated DNA from rat liver. *Exp. Cell Res.* 128:466–470.
40. Peters, K. E., and D. E. Comings. 1980. Two-dimensional gel electrophoresis of rat liver nuclear washes, nuclear matrix and HnRNP proteins. *J. Cell Biol.* 86:135–155.
41. Prescott, D. M., D. Myerson, and J. Wallace. 1972. Enucleation of mammalian cells with cytochalasin B. *Exp. Cell Res.* 71:480–485.
42. Robinson, S. I., B. D. Nelkin, and B. Vogelstein. 1982. The ovalbumin gene is associated with the nuclear matrix of chicken oviduct cells. *Cell* 28:99–106.
43. Ross, D. A., R.-W. Yen, and C-B. Chae. 1982. Association of globin ribonucleic acid and its precursors with the chicken erythroblast nuclear matrix. *Biochemistry.* 21:764–771.
44. Shaper, H. H., D. M. Pardoll, S. H. Kaufmann, E. R. Barrack, B. Vogelstein, and D. S. Coffey. 1980. The relationship of the nuclear matrix to cellular structure and function. *Adv. Enzyme Reg.* 17:213–248.
45. Shelton, K. R., L. L. Higgins, D. L. Cochran, J. J. Ruffolo, and P. M. Egle. 1980. Nuclear lamins of erythrocyte and liver. *J. Biol. Chem.* 255:10978–10983.
46. Small, D., B. Nelkin, and B. Vogelstein. 1982. Nonrandom distribution of repeated DNA sequences with respect to supercoiled loops and the nuclear matrix. *Proc. Natl. Acad. Sci. USA.* 79:5911–5915.
47. van Eeckelen, C. A. G., and W. J. van Venrooij. 1981. HnRNA and its attachment to a nuclear protein matrix. *J. Cell Biol.* 88:554–565.