A CHEMICAL METHOD FOR THE ISOLATION OF HeLa CELL NUCLEI AND THE NUCLEAR LOCALIZATION OF HeLa CELL ALKALINE PHOSPHATASE

M. J. GRIFFIN, R. P. COX, and N. GRUJIC. From the Department of Medicine, New York University School of Medicine, New York. Dr. Griffin's present address is Oklahoma Medical Research Foundation, Oklahoma City

Several chemical methods for the isolation of cell nuclei have recently been described. Calf thymus nuclei have been isolated by mild hypotonic shock followed by Ficoll gradient purification (1). A nonaqueous technique has been employed by Hammel and Bessman for purifying duck erythrocyte nuclei (2). Cell nuclei from cat brain have been obtained by using a detergent, Triton X-100, which results in a 2.8-fold enrichment of nuclei (as measured by the increase in the ratio of DNA/protein) (3). Triton X-100 has also been used to isolate rat liver nuclei, and in this preparation about 5-fold enrichment was obtained (4). Citric acid at pH 3.5 has been used to prepare HeLa cell nuclei, which were reported to appear uncontaminated by cytoplasmic material under the phase microscope (5).

In the present report the isolation of HeLa cell nuclei with sodium deoxycholate is described. The purity of the nuclei was evaluated by comparing the DNA-to-protein ratios of the whole cells to those of the separated cell fractions (6). The activity of β-glucuronidase was used as a measure of the lysosomal contamination of the various cell fractions. Assays of these cell fractions revealed that most of the alkaline phosphatase activity was associated with the nuclear fraction.

MATERIALS AND METHODS

Cell Cultures and Media

The methods and media used in this laboratory for monolayer and suspension cultures have been described (7, 8). Two different HeLa cell lines were studied: HeLa Ch, a cell line having a high constitutive level of alkaline phosphatase, and HeLa S_3, a line with low alkaline phosphatase activity which can be increased from 3- to 20-fold by growth in medium containing prednisolone (Δ^1-hydrocortisone) (7). In each experiment carried out with HeLa S_3 cells, replicate cultures were grown in medium with and without 1.0 μg/ml prednisolone phosphate. Monolayer cultures were harvested after 70 hr growth. The cells were removed from the glass surfaces by brief treatment with a solution of 0.025% trypsin (Microbiological Associates, Inc., Bethesda, Md., 1:200 Trypsin solution) and 3×10^{-4} M ethylenediamine tetraacetic acid (EDTA) as previously described (7). The action of trypsin and EDTA was stopped by suspension in growth medium.

Nuclear Isolation Technique

All procedures were carried out at 0°C. The cell suspension obtained from a Roux bottle ($8.0 \pm 0.2 \times 10^7$ cells) was centrifuged at 900 g (International Refrigerated Centrifuge, Yoke No. 253, International

Equipment Co., Needham Heights, Mass.) for 10 min, and the pellets were washed once with cold saline. The cell pellet was then resuspended in 15 ml of ice-cold 0.8% saline containing 10^{-3} M Tris hydrochloride, pH 7.4 (ST buffer). A 2.5 ml aliquot of whole cells was removed for assay of protein, RNA, DNA, and the activities of several enzymes. An ice-cold solution of 0.2% sodium deoxycholate in ST buffer (12.5 ml) was added slowly to the remaining 12.5 ml of cell suspension with gentle agitation with a glass rod. The resulting suspension was stirred 3 or 4 times during an 8 min period and then centrifuged at 900 g for 9 min. The supernatant or "cytoplasmic solution" was decanted and the "nuclear" pellet taken up in 10 ml of ST buffer (nuclei = 6.6 ± 0.8 × 10^7). The difference between the total number of cells used and the number of nuclei recovered probably reflects nuclear clumping, which interferes with accurate nuclear counts although a small amount of nuclear lysis cannot be excluded. Examination of the nuclear preparation by light and phase microscopy shows the nuclei to be free of observable cytoplasmic tabs. Whole cells and nuclei were also fixed in 90% methanol–10% formalin and dried with gentle heating on glass slides which were previously dipped in water. Cells and nuclei were stained either with Giemsa's or histochemically for alkaline phosphatase as previously described (7).

Assay Procedures

Since it has been found that high concentrations of deoxycholate interfere with the diphenylamine reaction for DNA (9), the whole cell aliquot and the nuclear suspension were divided into two portions. The first portion was treated with an equal volume of 1% sodium deoxycholate and used for enzyme assays (10). The second portion was treated with an equal

volume of 30% trichloroacetic acid and the precipitate sedimented at 1500 g. The precipitate was digested in 0.5 N perchloric acid at 75°C for 30 min, and the solution was clarified by centrifugation and assayed for DNA (9) and RNA (11). Calf thymus DNA and yeast RNA were used as standards. Alkaline phosphatase, acid phosphatase, and β-glucuronidase were assayed spectrophotometrically as previously described (10).

RESULTS

Fractionation of HeLa Ch cells was performed in 10 separate experiments. Table I summarizes the data from one experiment. It is apparent that alkaline phosphatase activity is almost totally confined to the nuclear fraction and that the increase in its specific activity (alkaline phosphatase activity/milligram of protein) parallels the enrichment factor for DNA (DNA content/milligram of protein). In all experiments, purification of alkaline phosphatase and enrichment of DNA were greater than 3.0-fold. The average purification for alkaline phosphatase in the 10 experiments was about 3.5-fold, and the enrichment of DNA about 4-fold. β-Glucuronidase assays performed on all cell fractions indicated that the nuclear fractions had less than 5 to 10% of the total cell enzyme activity. The low RNA content of the isolated nuclei is presumably a reflection of the purity of the nuclear fraction. A small amount of the total cell acid phosphatase activity was consistently found in the nuclear fraction. However, about 85% of the total cell acid phosphatase activity appeared in the cytoplasmic fraction.

TABLE I

Distribution of Phosphatases, DNA, and RNA in Subcellular Fractions Prepared from HeLa Ch Cells

Fraction	Alkaline phosphatase			Acid phosphatase		DNA			RNA		Protein
	Total activity*	Specific activity‡	Enrichment factor§	Total activity*	Specific activity‡	DNA	mg DNA / mg protein	Enrichment factor‖	RNA	mg RNA / mg protein	
						mg			mg		mg
Cell	575	20.8	1	3.0	0.110	7.5	0.28	1	18	0.66	22
Cytoplasm	50	5.0	0.5	2.6	0.260	—¶	—	—	10	1.00	11
Nuclei	460	66.3	3.3	0.9	0.130	5.5	0.78	2.8	1.8	0.26	7

* μmoles of p-nitrophenyl phosphate hydrolyzed/15 min.
‡ μmoles of p-nitrophenyl phosphate hydrolyzed/mg of protein.
§ Fold increase of alkaline phosphatase/mg of protein.
‖ Fold increase of DNA/mg of protein.
¶ Deoxycholate interference with assay.

TABLE II

Distribution of Alkaline Phosphatase, DNA, and RNA in Subcellular Fractions Prepared from HeLa S_3 Cells

Fraction	Alkaline phosphatase			DNA			RNA		Protein
	Total activity*	Specific activity‡	Enrichment factor§	DNA	mg DNA / mg protein	Enrichment factor||	RNA	mg RNA / mg protein	
				mg			mg		mg
Cells grown without prednisolone									
Cell	17.4	0.91	1	5.6	0.31	1	8.0	0.4	19
Cytoplasm	3.6	0.21	0.2	—¶	—	—	—	—	17
Nuclei	18.6	6.60	7	3.2	1.15	3.8	1.6	0.6	3
Cells grown with prednisolone									
Cell	43.8	2.80	1	4.8	0.31	1	16.5	1.1	16
Cytoplasm	8.0	0.60	0.2	—¶	—	—	—	—	13
Nuclei	27.2	5.50	2	3.5	0.73	2.3	2.0	0.4	5

*, ‡, §, ||, ¶ See legend for Table I.

Table II summarizes findings from one of four experiments on the isolation of nuclei from HeLa S_3 cells grown in the presence (induced) and absence (control) of prednisolone. The results obtained on HeLa S_3 cells grown in suspension cultures were similar to those obtained on cells in monolayer cultures. For both uninduced and induced HeLa S_3 cells, the enrichment of DNA closely parallels the purification of alkaline phosphatase in the nuclear fraction (Table II). In uninduced HeLa S_3 cells, the amount of enrichment of DNA and the purification of alkaline phosphatase were similar to those seen in HeLa Ch cells. Induced HeLa S_3 cells, however, showed less enrichment of both DNA and alkaline phosphatase. This finding is probably a result of a hormone-induced alteration in the cell membrane which renders it more resistant to deoxycholate lysis (12). The biochemical data agreed with cytological observations. The nuclear preparations appeared to consist of at least 95% nuclei by light and phase microscopy. None of the nuclei had observable adhering cytoplasm. Histochemical studies of the intact cells for alkaline phosphatase showed that virtually all of the enzyme activity was located in the nucleus and that the nucleoli stained intensely. Following nuclear isolation, the nuclei retained the alkaline phosphatase histochemical activity.

DISCUSSION

The results indicate that the nuclear fraction obtained from HeLa cells by a simple chemical method employing 0.1% sodium deoxycholate is relatively free of cytoplasmic material. The lipophilic properties of this anionic cholic acid derivative solubilize most of the cytoplasm and leave the nucleus intact. The primary advantages of this method are its simplicity and reproducibility. Deoxycholate has been used previously for separating microsomal membranes and ribosomes from the microsomal fraction of rat liver (13) and rat kidney cells (8). Recently, mechanically prepared HeLa nuclei were freed of adhering cytoplasmic contaminants by brief treatment in a solution of 0.5% sodium deoxycholate and 1.0% of the nonionic detergent, Tween 40 (14).

Histochemical studies have shown that alkaline phosphatase activity is present in the nuclei of most animal and plant cells (7, 15). The presence of alkaline phosphatase in the nuclei of animal cells in culture and its concentration on the chromosomes during cell division have been challenged as diffusion artifacts which arise during the staining procedure (15). However, the results obtained in this study which indicate that most of the alkaline phosphatase is found in the nuclear fraction of these HeLa cells support the histochemical evidence for nuclear localization of this enzyme.

The physiological basis for nuclear localization of alkaline phosphatase is not known. However, since mammalian alkaline phosphatase has been shown to have inorganic pyrophosphatase activity (16), it is possible that this enzyme could drive the reversible DNA and RNA synthesizing

reactions to completion by cleaving inorganic pyrophosphate which is one of the products of these reactions.

SUMMARY

A simple, rapid chemical technique for isolating HeLa cell nuclei is described. The nuclei are relatively pure as judged by biochemical and histochemical observations. Alkaline phosphatase is located predominately in the nucleus of HeLa cells.

REFERENCES

1. ALLFREY, V. G., V. C. LITTAU, and A. E., MIRSKY. 1964. Methods for the purification of thymus nuclei and their application to studies of nuclear protein synthesis. *J. Cell Biol.* **21**:213.

2. HAMMEL, E. L. and S. P. BESSMAN. 1964. Hemoglobin synthesis in avian erythrocytes. *J. Biol. Chem.* **239**:2228.

3. 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.

4. 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.

5. STUDZINSKI, G. P., and R. LOVE. 1964. Effects of puromycin on the nucleoproteins of the HeLa cell. *J. Cell Biol.* **22**:493.

6. ROODYN, D. B. 1963. *In* Methods of Separation of Subcellular Components. J. D. Grant, editor. Cambridge University Press, London. 20 and Plate VIa.

7. COX, R. P., and C. M. MACLEOD 1962. Alkaline phosphatase content and the effects of prednisolone on mammalian cells in culture. *J. Gen. Physiol.* **45**:439.

8. GRIFFIN, M. J., and R. P. COX. 1966. Studies on mechanism of hormonal induction of alkaline phosphatase in human cell cultures. I. Effects of puromycin and actinomycin D. *J. Cell Biol.* **29**:1.

9. BURTON, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**:315.

10. GRIFFIN, M. J., and R. P. COX. 1964. Subcellular localization of rat kidney alkaline phosphatase. *Nature.* **204**:476.

11. ASHWELL, G. 1962. *In* Methods in Enzymology. S. P. Colowick and N. O. Kaplan editors Academic Press, Inc., New York. **3**:87.

12. MELYNKOVYCH, G. 1966. Glucocorticoid-induced resistance to deoxycholate lysis in HeLa cells. *Science.* **152**:1086.

13. ERNSTER, L., P. SIEKEVITZ, and G. PALADE. 1962. Enzyme-structure relationships in the endoplasmic reticulum of rat liver. *J. Cell Biol.* **15**:541.

14. PENMAN, S. 1966. RNA metabolism in the HeLa cell nucleus. *J. Mol. Biol.* **17**:117.

15. DANIELLI, J. F. 1953. *In* Cytochemistry, A Critical Approach. John Wiley & Sons Inc., New York 50-63.

16. COX, R. P., and M. J. GRIFFIN. 1965. Pyrophosphatase activity of mammalian alkaline phosphatase *Lancet. II*:1018.