A SIMPLE AND RAPID METHOD FOR THE ISOLATION OF ENZYMATICALLY ACTIVE HELA CELL NUCLEI

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Since HeLa cells are one of the most commonly studied established cell lines, there is a need for a simple and rapid method for isolating enzymatically active cell nuclei which are not aggregated and thus can be pipetted in reproducible aliquots. We report here such a procedure which may be used for both large and small volumes of suspension cultures and which requires no specialized equipment. The method is a modification of previous techniques (1–3), all of which are based upon the use of Triton detergents. Although several other procedures for isolating HeLa cell nuclei have been published (4–7), the convenience and utility of the present method warrant consideration.

MATERIALS AND METHODS

HeLa cells, type S3-1 (initially supplied by Dr. Bernard Moss, National Institutes of Health, Bethesda Md.) were grown, in spinner flasks, in Eagle's minimum essential medium (8), supplemented with 5% horse serum, 4 mM glutamine, and 100 u/ml each of penicillin and streptomycin.

Solutions used for cell fractionation were as follows: solution 1, 0.32 M sucrose, 0.002 M $MgCl_2$, 0.001 M potassium phosphate, pH 6.8; solution 2, 0.01 M NaCl, 0.001 M potassium phosphate, pH 6.8; solution 3, 0.32 M sucrose, 0.001 M $MgCl_2$, and 0.3% Triton N-101, adjusted to pH 6.2–6.4 with 0.001 M potassium phosphate.

Sources of materials were as follows: polyadenylic acid, Miles Laboratories, Inc., Elkhart, Ind.; CTP-^{3}H Schwarz Bio Research Inc., Orangeburg, N. Y.; Dounce homogenizers, Blaessig Glass Specialties, Rochester, N. Y. Nuclear exoribonuclease was assayed according to the method of Lazarus and Sporn (3), and RNA polymerase according to the method of Widnell and Tata (9). DNA was determined by the method of Burton (10), and RNA by the method of Scott et al. (11).

For electron microscopy the nuclei were fixed with

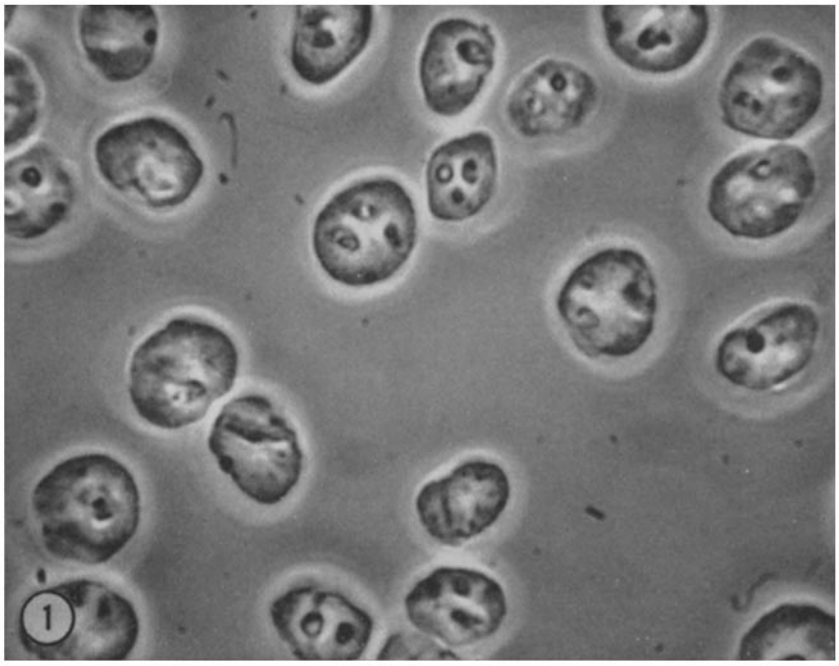

FIGURE 1 Phase-contrast photomicrograph of isolated nuclei suspended in solution 1. × 960.

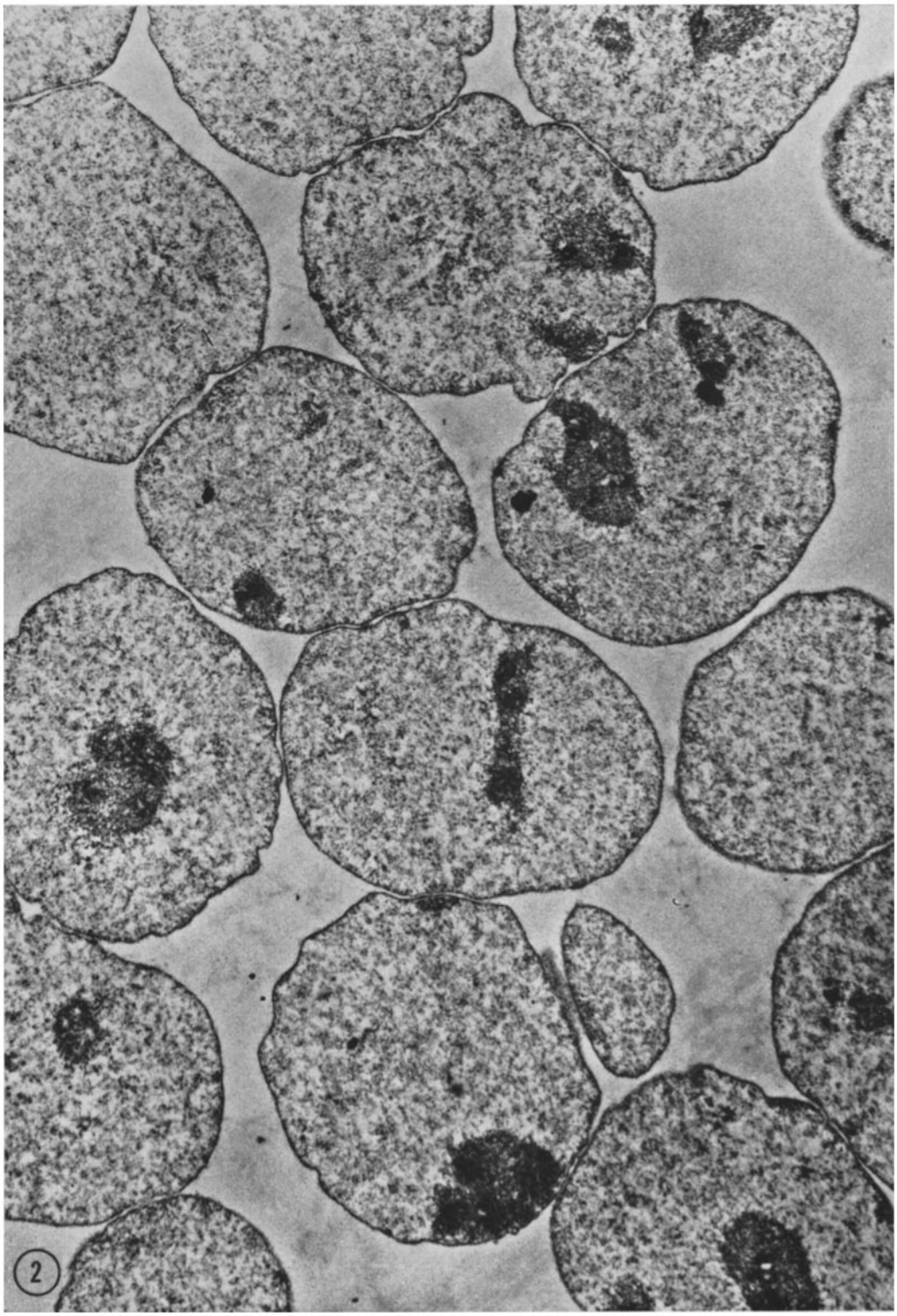

FIGURE 2 Low-power electron micrograph of an ultrathin section of isolated nuclei. The specimen was double stained with uranyl acetate and lead citrate. × 10,000.

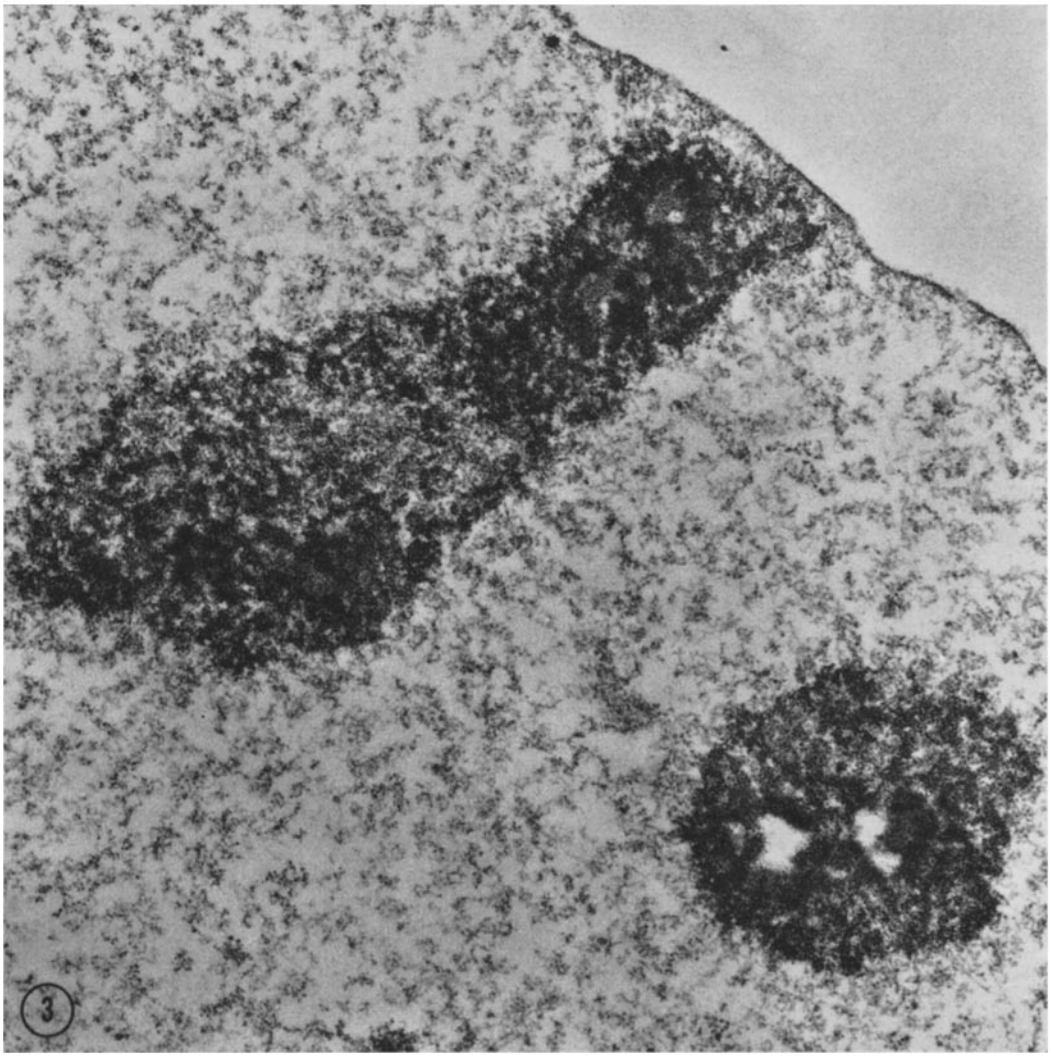

FIGURE 3 High-power electron micrograph of an isolated nucleus, showing complete removal of the outer layer of the nuclear envelope and the intact structure of the nucleolus. The condensed chromatin shows slightly reduced electron opacity. × 27,300.

2.5% glutaraldehyde and 1% osmium tetroxide in *s*-collidine buffer for 1 hr each, dehydrated with graded acetone, and embedded in Epon-Araldite. The ultrathin sections were double stained with uranyl acetate and lead citrate for 15 min each. Photographs were taken with a Philips 200 electron microscope with direct magnification of 1680–9100.

Preparation of Nuclei

The entire cell fractionation procedure was performed at 0°C. HeLa cells (approximately 4 × 10^5/ml) were harvested by centrifugation at 1000 *g* for 6 min. The cell pellet was washed with large volumes (50 ml/10^8 cells) of solution 1, recentrifuged, and suspended in a similar volume of solution 2 for 15 min. The suspension was then centrifuged at 800 *g* for 10 min, the pellet was suspended in solution 3 (25 ml/10^8 cells) in a Dounce homogenizer and was treated with 10 passes of a loose and tight pestle. The homogenate was centrifuged at 800 *g* for 10 min, and the supernatant was discarded; the pellet was resuspended in solution 3, treated again with both pestles, and recentrifuged. The final nuclear pellet can be suspended easily in solution 1 to give an

TABLE I

RNA Polymerase Activity in Isolated HeLa Cell Nuclei

Reaction mixture	CMP incorporated per 10^7 nuclei
	μμmoles
Complete	576
Complete, no incubation	9
Complete, + actinomycin D (20 μg/ml)	159
Minus UTP	52
Minus ATP, GTP, UTP	13
Minus nuclei	2

The reaction mixture contained the following in a volume of 1.0 ml: 10^7 nuclei in 0.3 ml of solution 1; Tris-Cl, pH 8.0, 100 μmoles; $(NH_4)_2SO_4$, 400 μmoles; $MnCl_2$, 4 μmoles; ATP, GTP, UTP, 0.6 μmole each; CTP, 0.04 μmole containing 2 μCi CTP-5-^{3}H. The reaction mixture was incubated for 20 min at 37°C, and the reaction was terminated with 2.0 ml of ice-cold 1.0 M perchloric acid (PCA). The precipitates were transferred to Whatman GF/C glass filters and washed four times with 5 ml of ice-cold 0.2 M PCA. The filters were counted in a liquid scintillation counter after the addition of 1 ml of NCS reagent and 15 ml of scintillator. Values reported are the average of at least six determinations.

unaggregated preparation. DNA determinations on the initial suspension cultures and the final pellets indicated that the yield of nuclei was from 70 to 80%.

RESULTS AND DISCUSSION

Fig. 1 shows a representative phase-contrast photomicrograph of the resuspended final nuclear pellet, and Figs. 2 and 3 show two electron micrographs. Virtually no cytoplasmic components and no unbroken cells were seen in any of the preparations. Electron micrographs show the isolated nuclei to be smooth in outline, with the outer layer of the nuclear envelope completely removed. The chromatin network is well preserved, although the condensed chromatin appears to have a slightly reduced electron opacity. The nucleoli consist of dense granular and fibrillar components, essentially identical with those seen in intact cells.

Nuclei prepared by the method described above had high activities of both RNA polymerase and the nuclear exoribonuclease first described in the Ehrlich ascites tumor (3). RNA polymerase activity is reported in Table I; the level of activity is of the same order of magnitude as that reported by other investigators for nuclei prepared from rat liver (9). Nuclear exoribonuclease was assayed under standard conditions (3) with polyadenylic acid as the substrate; adenosine-5′-monophosphate was virtually the sole product of the reaction and was identified by thin-layer chromatography and cleavage to adenosine by snake venom (3). The amount of exoribonuclease activity was found to be 4.5 u/mg nuclear DNA. The RNA/DNA ratio of the isolated nuclei is 0.24. Triton N-101 inactivates mitochondrial oxidative enzymes in HeLa cell homogenates, and hence an assay for cytochrome oxidase could not be performed on isolated nuclei. However, no mitochondrial profiles were seen in careful electron microscopic examination of multiple preparations of nuclei.

SUMMARY

A simple and rapid method for the isolation of nuclei from HeLa cells is reported. This method is suitable for either large or small volumes of cell cultures, requires no specialized equipment, and yields nuclei which are not aggregated and which can be easily resuspended in an individually dispersed state. The nuclei are free of unbroken cells and cytoplasmic components, have a well-defined ultrastructure, and have high RNA polymerase and exoribonuclease activities.

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