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Reconstruction of Mammalian Cells from Nuclear and Cytoplasmic Components Separated by Treatment with Cytochalasin B

(latex spheres/cytoplasmic labels/Sendai virus)

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ABSTRACT **Mouse L929 cells were separated into enucleated cytoplasmic components (cytoplasts) and nucleated subcellular fractions (karyoplasts) in the presence of cytochalasin B. Karyoplasts from cells containing tritiated nuclei were fused, using inactivated Sendai virus, to cytoplasts from cells containing large (1.0-μm diameter) latex spheres in the cytoplasm. Mononucleated cells containing radioactive nuclei and large latex spheres in the cytoplasm were observed among the products of the fusion reaction. Some of these cells were in mitotic configurations. The results indicate that cells capable of undergoing mitosis can be reconstructed from the products of cellular enucleation in the presence of cytochalasin B.**

Cytochalasin B causes the nucleus of animal cells growing as monolayers in culture to segregate into an outpocketing of cytoplasm on the top of the cell (1). The process frequently develops to such an extent that the outpocketing remains attached to the main body of the cytoplasm only by a thin cytoplasmic stalk. Occasionally the stalk breaks, and the cell becomes separated into nucleate and enucleate portions (1). The frequency of this separation into nuclear and cytoplasmic parts can be greatly increased by pulling the nucleus away from the cell with centrifugal force while the cell is exposed to cytochalasin B (2, 3).

The enucleated cells (cytoplasts) quickly recover from the distortion produced by cytochalasin and remain intact for 1–3 days (4). These cytoplasts continue to synthesize proteins for at least 12 hr (2) and can support synthesis activities of viruses (5, 6). Cytoplasts can also be trypsinized and replated on a new substrate (7).

The nuclei removed from cells by the cytochalasin-centrifugation technique are surrounded by a thin shell of cytoplasm and a plasma membrane (8). These nucleated structures (karyoplasts) contain ribosomes, occasional mitochondria, and a few fragments of the endoplasmic reticulum (8), but always lack centrioles, microtubules, and a Golgi apparatus (4). Karyoplasts remain intact for a least 24 hr and continue to synthesize RNA for several hr after the enucleation procedure.

Using inactivated Sendai virus as a fusing agent, it should be possible to reconstruct a whole cell by joining a cytoplast with a karyoplast. Development of such a technique would permit the construction of new cells in which the nucleus is derived from one parental cell type and the bulk of the cytoplasm is derived from another cell type. Such "hybrids" would be useful for analyzing nuclear–cytoplasmic interactions on a variety of cellular activities involved in the cell life cycle, cell aging, differentiation, and cell transformation.

We have developed a procedure for the reconstruction of mouse L cells from nuclear and cytoplasmic parts (karyoplasts and cytoplasts). Such reconstructed cells are viable as judged by their ability subsequently to enter mitosis. The reconstruction method is described in this paper.

Abbreviations: EMEM, Eagle's Minimal Essential Medium; EBSS, Earle's Balanced Salt Solution.

MATERIALS AND METHODS

Mouse L929 cells were grown in Eagle's Minimal Essential Medium (EMEM) (Gibco, Grand Island, N.Y.) supplemented with 10% fetal-bovine serum (Flow Labs, Inc., Rockville, Md.), 50 IU/ml of penicillin, and 50 μg/ml of streptomycin sulfate. Cells to be enucleated were planted on plastic coverslips punched from the bottom of Falcon plastic tissue-culture dishes (Falcon Plastics, Oxnard, Calif.) and were enucleated by techniques described elsewhere (9).

Procedures. Cells that served as cytoplast donors were grown in EMEM containing 10^6/ml of large latex spheres (1.0-μm diameter) (Polysciences, Inc., Warrington, Pa.). Each cell took in by phagocytosis usually 5 to 10 spheres, but of the total population about 6% of the cytoplasts had no spheres and about 6% had more than 20.

During enucleation, almost all of the spheres remained with the cytoplasts and served, therefore, to mark the source of the cytoplasm in subsequently reconstructed cells. To obtain as pure a population of cytoplasts as possible, the cells were subjected to the enucleation procedure twice. The percentage of cells enucleated after one treatment was greater than 90% and after the second treatment was greater than 99%.

Cells used to provide karyoplasts were incubated in EMEM containing 0.2 μCi/ml [^{3}H]thymidine (20 Ci/mM, New England Nuclear, Boston, Mass.) and 5×10^6/ml of small latex spheres (0.5-μm diameter) (Fullam, Inc., Schenectady, N.Y.) for 48 hr. Essentially all of the nuclei were labeled with [^{3}H]thymidine. These nuclei could, therefore, subsequently be distinguished autoradiographically from the few nuclei in the whole cells that remained as contaminants of the cytoplast preparation. Because almost all the small latex spheres remain with the cytoplast during enucleation, most of the resulting karyoplasts contain no spheres. The cytoplasts (to be discarded) in this case contained an average of about 20 spheres and, of the total population, only 0.5% contained no spheres. As a consequence, any whole cells that detached from the coverslip during centrifugation could be identified

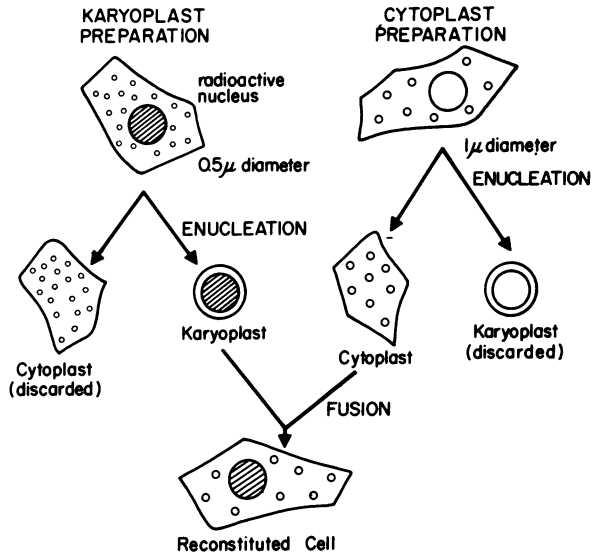

FIG. 1. Schematic representation of the experimental design. Karyoplasts were derived from cells labeled with [^{3}H]thymidine and small (0.5-μm diameter) latex spheres and fused to cytoplasts from cells labeled only with large (1.0-μm diameter) latex spheres. The latex spheres permitted recognition of the source of the cytoplasm. Mononucleated cells with radioactive nuclei and only large latex spheres in the cytoplasm were considered as cells reconstructed from components derived from different sources. Fusions resulting from whole cell contaminants in the karyoplast preparation were recognized by the numerous small spheres in the cytoplasm and were excluded from consideration as reconstructed cells.

in subsequent fusion experiments by the presence of numerous small spheres.

Plan of Experiment. The plan for the entire experiment is shown in Fig. 1. The cytoplasts contain large latex spheres (1.0-μm diameter) while the karyoplasts contain none, or occasionally one to five small ones (0.5-μm diameter). The nuclei of the few whole cells that contaminate the cytoplast preparation are not radioactive, while essentially all karyoplast nuclei contain [^{3}H]thymidine incorporated into DNA. Thus, the product of a fusion between a karyoplast and a cytoplast will contain a labeled nucleus and a cytoplasm with five to ten large latex spheres and no small spheres or occasionally one to five small spheres. Any whole cell contaminants (and their subsequent fusion products) in the original cytoplast preparation can be identified by the presence of an unlabeled nucleus. Any whole cell contaminants (or their subsequent fusion products) in the original karyoplast preparation can be identified by the presence of multiple small latex spheres in the cytoplasm.

Fusion of Karyoplasts and Cytoplasts. The cytoplasts were removed from coverslips by treatment for a few minutes with 0.25% trypsin, washed twice with Earle's Balanced Salt Solution (EBSS) by centrifugation (1000 $\times$ g), and finally resuspended in EBSS. The karyoplasts were washed twice by centrifugation (1000 $\times$ g) in EBSS and finally resuspended in EBSS. The numbers of karyoplasts and cytoplasts were determined on small aliquots in a hemocytometer. To carry out fusion, approximately 8 $\times$ 10^5 cytoplasts and karyoplasts were mixed together in a centrifuge tube and centrifuged at 1000 $\times$ g to form a pellet. The cell parts were resuspended in 0.1 ml of ice cold EBSS, and 2 aliquots of 10 μl each were withdrawn and plated onto 22-mm square coverslips in 35-mm petri dishes. These aliquots served as "0" time controls for virus-induced fusion. Inactivated Sendai virus (Connaught Labs, Toronto, Canada) was added (0.05 ml; 10,000 hemagglutination units/ml) to the centrifuge tube and the mixture was incubated at 0° for 5 min and then at 37° for 15 min. Another 0.05 ml of virus was added and the incubations at 0° and 37° were repeated. The mixture was split into 2 aliquots and each was added to a 35-mm petri dish containing a glass coverslip. The cells in the four petri dishes were incubated overnight at 37° and then fixed with 3:1 (alcohol: acetic acid) and coated with NTB2 (Eastman Kodak Co., Rochester, N.Y.) autoradiographic emulsion. After 14 days, a "0" time and a "fusion" coverslip were developed, stained with crystal violet, and examined for fused cytoplasts and karyoplasts. The remaining coverslips were developed, stained, and examined after 30-days exposure.

RESULTS AND DISCUSSION

The "0" time coverslips (cytoplasts and karyoplasts mixed but without Sendai virus) were used to determine the amount of whole cell contamination in the cytoplast and karyoplast preparations and the amount of spontaneous fusion. No fusion products were detectable. Karyoplasts that attach to the coverslip remain as spherical or ellipsoidal, radioactive bodies (Fig. 2*a*), occasionally with one to five small latex spheres. Whole cells in this preparation attach, flatten out, and contain latex spheres of either size. Approximately 6% of the radioactive nuclei in the karyoplast preparation were in whole cells. The cytoplast preparation was contaminated by less than 1% with nucleated cells.

A few cells on the fusion slides were whole cell contaminants derived from the karyoplast and cytoplast preparations. These were distinguished as cells with radioactive nuclei and multiple small spheres (contaminants among the karyoplasts) and cells with nonradioactive nuclei and large spheres (contaminants among the cytoplasts). Also present were mononucleated and multinucleated cells containing both large and small beads (Fig. 2*b*); these could represent several possible fusion combinations but always involved a whole-cell contaminant from the karyoplast preparation. From the standpoint of our objective, the most important kind of cell on the fusion slide contained a single, radioactive nucleus and large latex spheres but no small ones. A cell of this type is shown in Fig. 2*c*. In this experiment, 42% of the karyoplasts on the slide had fused to form cells, most of which were multinucleated. Of the karyoplasts that fused, 13% (or about 5% of the total) formed the desired uninucleated cells, an example of which is shown in Fig. 2*c*. It is possible that some of these were formed by the fusion of a single karyoplast with more than one cytoplast. More than 200 reconstructed cells of the type in Fig. 2*c* were unequivocally identifiable. Some of the reconstructed cells believed to have formed by fusion of a single karyoplast with one or more cytoplasts entered mitosis (Fig. 2*d* and *e*), providing evidence that reconstructed cells are capable of the resumption and completion of the cell cycle. This indicates that reconstructed cells are capable of proliferation.

It is possible that the kind of reconstructed cell shown in Fig. 2*c* could have formed by fusion between a whole-cell

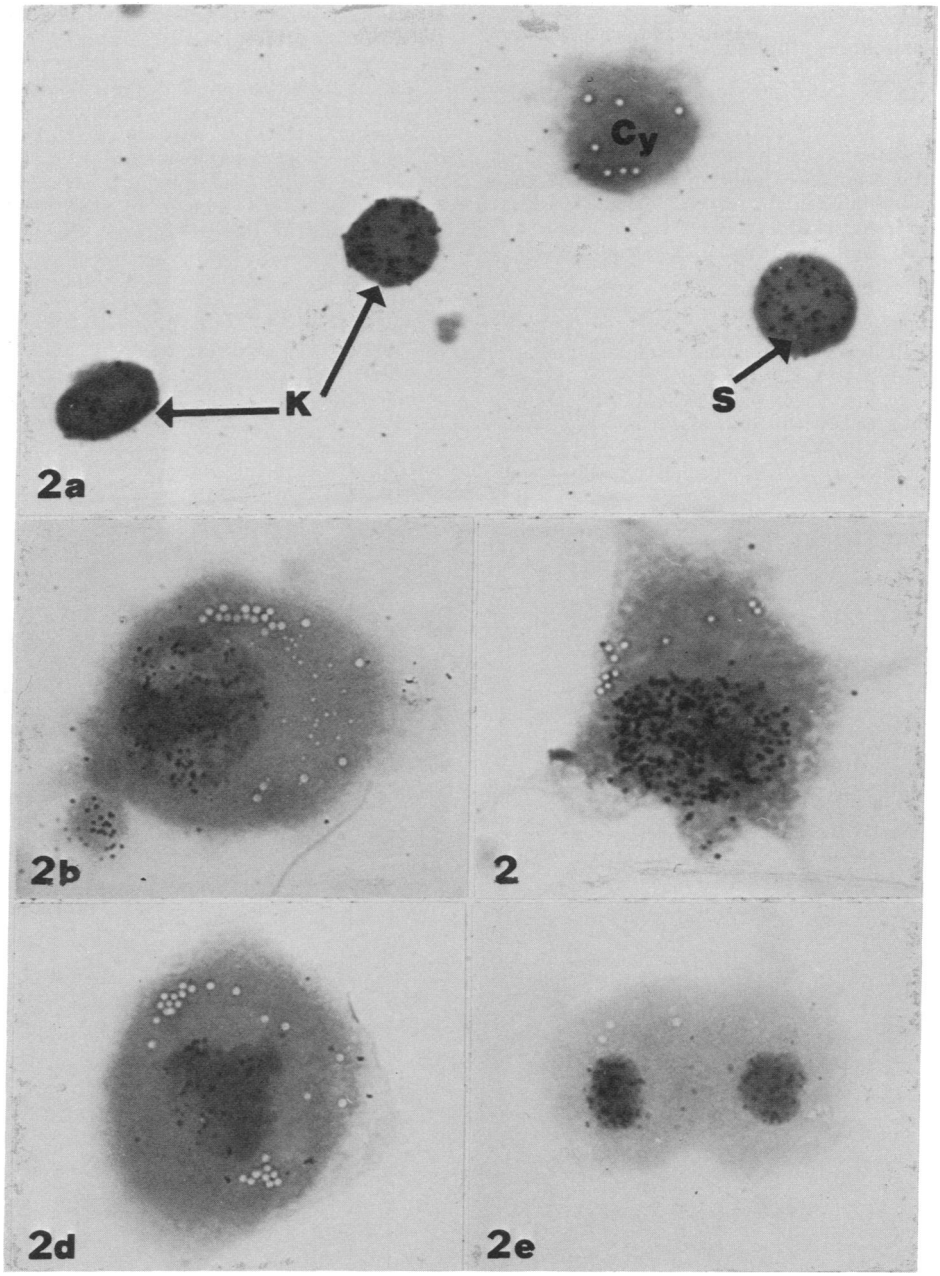

FIG. 2. Photomicrographs of separated L929 cell components and products of the fusion reaction. (2*a*) Cytoplasts (Cy) containing large latex spheres attach and flatten out while karyoplasts (K) remain as spherical or ellipsoidal radioactive bodies, occasionally containing a small latex sphere (S). ×1000. (2*b*) Cell formed by fusion of a whole-cell contaminant, in a karyoplast preparation, to a cytoplast. Note the presence of latex spheres of both sizes. ×1200. (2*c*) A reconstructed cell, indicated by a radioactive nucleus and only large latex spheres in the cytoplasm. ×1000. (2*d* and 2*e*) Reconstructed cells in metaphase (2*d*, ×1200) and telophase (2*e*, ×1000), indicating that the constructed cells are viable.

contaminant in the karyoplast preparation and a cytoplast. This could be the case provided the whole-cell contaminant was one of the few cells that had failed to be marked by small latex spheres before preparation of the karyoplasts. We should expect no more than 10 such fusion events on the basis of the following calculation. In the karyoplast preparation there were 4.8×10^4 whole-cell contaminants (6% of the 8×10^5 karyoplasts used). Among these whole-cell contaminants, 0.5%, or 240, had failed to take up the small latex spheres. Of these, 20% were withdrawn for the "0" time control slides. Of the 192 still remaining, we assume that 80 fused because 42% of the nuclei in the karyoplast preparation participated in fusion events. Of these 80, we would expect 13%, or 10 cells, to fuse with a cytoplast to form uninucleated cells that would contain a radioactive nucleus and only large latex spheres in the cytoplasm. We observed more than 200 with labeled nuclei and only large spheres and conclude that most of these could not have formed by fusion of a whole cell with a cytoplast, but represent instead a cytoplast–karyoplast recombinant.

In summary, the experiments show that whole cells can be reconstructed from the nuclear and cytoplasmic parts separated by the cytochalasin-centrifugation technique. This

establishes the feasibility of constructing mixed cell types by combining karyoplasts and cytoplasts derived from parental cells of different types.

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