The Surface Morphology and Fine Structure of CHO (Chinese Hamster Ovary) Cells Following Enucleation
(cytoschalasin B/karyoplasts/cytoplasts)

J. W. SHAY, K. R. PORTER, AND D. M. PRESCOTT

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colo. 80302

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ABSTRACT Chinese hamster ovary cells grown in monolayer culture and exposed to cytoschalasin B were enucleated by centrifugation. Thereafter, the karyoplasts (the nucleated parts obtained from the bottoms of the centrifuge tubes) and the cytoplasts (the enucleated cytoplasmic parts attached to the coverslips) were allowed to recover and subsequently were examined by scanning and transmission electron microscopy. Microscopy of thin sections revealed that the karyoplasts, limited by an intact plasma membrane, contain an intact nucleus surrounded by a layer of cytoplasm that includes ribosomes, mitochondria, and fragments of the endoplasmic reticulum, but no centrioles or microtubules. The cytoplasts, similarly examined, appear to contain all cytoplasmic organelles and systems, including centrioles and microtubules. The karyoplasts, when replated in fresh medium adhere to the substrate but remain essentially spherical and are incapable of motility. They disintegrate in about 72 hr. The cytoplasts, under identical conditions, recover a shape similar to that of the whole Chinese hamster ovary cell and display some motility. They generally survive not more than 48 hr. It appears that this enucleation procedure consistently separates the nucleus and limited cytoplasm from the centrosphere and microtubule-containing cytoplasts and, furthermore, that the form-determining and motility mechanisms reside in the cytoplast and function without nuclear participation for the short period of viability.

Cytoschalasin B causes rapid and dramatic changes in the morphology of vertebrate cells growing in monolayer culture (1). In some instances the nucleus is segregated into an outpocketing on the free surface of the cell and may remain attached to the main body of the cell only by a thin stalk of cytoplasm. Occasionally the stalk breaks resulting in a separation of the cell into nuclear and cytoplasmic components (1-3). This process can be made to occur in virtually all cells in a culture by using centrifugal force during treatment with cytoschalasin B so that the nucleus is pulled away from the cell (4, 5).

Thus, it becomes possible to separate more than 95% of the cells in a culture into nuclear and cytoplasmic parts (6, 7). Both parts recover quickly from the effects of cytoschalasin B. The nucleated part, called the karyoplast (8), consists of a nucleus enclosed in a thin shell of cytoplasm with an intact plasma membrane (9). The cytoplasmic part, called the cytoplast (8), contains the bulk of the cytoplasm and has representatives of all cytoplasmic organelles and systems including centrioles and microtubules. The karyoplasts continue to synthesize RNA and protein for at least several hours and remain intact for 48-72 hr. Cytoplasts synthesize protein for at least 12 hr and in this period can support metabolic activities of viruses (4, 6, 7). The cytoplasts survive for up to 48 hr.

In this paper we describe observations by scanning and transmission electron microscopy on the structure and behavior of karyoplasts and cytoplasts derived from a Chinese hamster ovary (CHO) cell line. These observations extend those in earlier reports (8,9) and establish a basic background in the experimental use of karyoplasts and cytoplasts for the analysis of nuclear-cytoplasmic interactions.

MATERIALS AND METHODS
Chinese hamster ovary cells were grown to semi-confluency on no. 1 thickness glass (round 18 mm) or plastic (25 mm) coverslips in Ham's F-12 medium containing 10% fetal calf serum. The plastic coverslips were punched out of the bottoms of Falcon plastic tissue culture dishes (Falcon Plastics, Ox nard, Calif.) with a heated piece of stainless steel tubing. The coverslips were then inserted cell-side down into centrifuge tubes containing 10 µg/ml of cytoschalasin B (Imperial Chemical Industries, Ltd., Great Britain) in Ham's F-12 and centrifuged in a Sorvall SS-34 prewarmed head as previously described (4). Glass coverslips were spun for 40 min at 3000 to 34000 X g at 37°, whereas the more sturdy plastic coverslips were spun at 17,000 X g for 20 min. After one centrifugation in cytoschalasin B the enucleation was usually greater than 95%. If the cells were allowed to recover from cytoschalasin B by incubation in fresh medium for approximately 20 min and then recentrifuged a second or third time in cytoschalasin B, the enucleation was increased to 99+% (7).

Since the cytoschalasin B was dissolved in absolute alcohol (1 mg/ml), control cells were centrifuged in F-12 medium without it or in F-12 medium containing 10 µl/ml of absolute ethanol. They were not enucleated.

After enucleation the cytoplasts (the cytoplasmic portions attached to the coverslips but without nuclei) were allowed to recover in fresh whole medium and fixed at various intervals for scanning and transmission microscopy. The karyoplasts (the nuclear fraction obtained from the bottoms of the centrifuge tubes) were resuspended in fresh medium and plated out on glass coverslips for fixation at various intervals. The preparation of cultured cells for scanning electron microscopy has been previously described (10). For transmission electron microscopy, monolayer cultures on glass coverslips were fixed by a modification of the procedures developed by Robbins and Gonatas (11). The sections were examined with a JEM 100 B or Philips 300 transmission electron microscope, and the cell surfaces were examined with a Cambridge S-4 scanning electron microscope.

Whole cells and cytoplasts were also examined with a JEM 1000 electron microscope. For this, cells were grown on formvar-coated coverslips and then enucleated as described above. After 2-5 hr at 37° the cytoplasts were fixed and formvar
Figs. 1 and 2. Scanning electron micrograph of a confluent culture of Chinese hamster ovary (CHO) cells (Fig. 1). CHO nuclear fraction containing a few whole cells but mostly karyoplasts. Some of the karyoplasts showing surface microvilli or blebs pile up on one another with no apparent contact effect (Fig. 2). X620 and X561.

Figs. 3, 4, and 5. CHO cytoplasts fixed after 30 min-, 24 hr-, and 48 hr-recovery in fresh medium. For the first 24 hr of recovery the surfaces are usually smooth or contain microvilli (Fig. 3) while after 24 hr the surface usually contain blebs (Fig. 4). By 48 hr the cytoplasts degenerate (Fig. 5). X1,600.

FIG. 6. Transmission electron micrograph of control CHO cells revealing the presence of such cytoplasmic organelles as Golgi bodies, centrioles, and microtubules which are not present in karyoplasts.

with cells attached were floated free from the coverslips [according to the techniques of Buckley and Porter (12)]. Selected areas were then picked up on grids, poststained in uranyl acetate, dehydrated, and finally dried by the critical point method. In this form they were examined by high voltage microscopy.

OBSERVATIONS

Scanning electron microscopy of CHO cells

The surface morphology of CHO cells as observed by scanning microscopy has been described previously (13, 14). It varies with each phase of the cell cycle in confluent cultures (13) and is influenced by contact with other cells (14). Ordinarily, in confluent cultures (Fig. 1), the G1 cell shows multiple blebs on its free surface which diminish in number towards the end of G1. During S, the cells spread out and are relatively smooth, showing only a small number of microvilli and occasional marginal ruffles. The cells in the cultures used in this study were in logarithmic growth and, as depicted in Fig. 1, were not synchronized. At the time of enucleation they were in either G1, S, or G2 since those in mitosis are detached by the centrifugal force used for enucleation and become part of the pellet containing the karyoplasts (see Fig. 9).

The surface morphology of the enucleated cytoplasts (cytoplasts)

Within a very short period after enucleation (approximately 30–60 min) the cytoplasts recover from the distortions imposed by cytochalasin and centrifugation and spread out...
Enucleation of CHO Cells with Cytochalasin B

Thinly on the original substrate (15). The resulting surfaces of cytoplasts are relatively smooth except for a few microvilli and there is little or no evidence of blebs (Fig. 3). After 30 min of recovery (Fig 3), the cytoplasts closely resemble in form and surface features cells in confluent cultures (Fig. 1). By the end of 24 hr the cytoplasts, though still thinly spread, begin to show surface excrescences including a number of blebs (Fig. 4). The cytoplasts generally retain this degree of integrity from 24 to 36 hr after enucleation. By 48 hr of incubation the cytoplasts begin to disintegrate and small strands of cytoplasm separate from the main mass (Fig. 5).

The surface morphology of the nucleated portion (karyoplasts)
The nucleated parts of CHO cells when replated on plastic or glass make contact with the substrate over a small area and spread out only slightly if at all (Fig. 2). Their surfaces usually remain covered with blebs or microvilli up to the time they disintegrate (about 72 hr). The small amount of cytoplasm associated with the nucleus after enucleation does adhere to the substrate and shows evidence of ruffling at the margins where the karyoplast is in contact with the substrate. If the karyoplasts are replated at very high densities, colonies frequently appear in which many karyoplasts pile up on one another with no apparent response to contact (Fig. 2). Some of these karyoplasts show multiple blebbing, or microvilli or both (Fig. 2).

Fine structure of complete CHO cells
The structure of CHO cells as observed by transmission microscopy of thin sections reveals the nucleus, and a cyto-

Figs. 7 and 8. Enucleated CHO cells (cytoplast) fixed after 3 hr of recovery in fresh medium (Fig. 7). At a higher magnification (Fig. 8) a centriole and associated microtubules are readily identified. X8,000 and X24,300.

Figs. 9 and 10. Electron micrographs of karyoplasts fixed after 2-hr recovery in fresh medium (Fig. 9). Fig. 10 reveals the substructure of the cytoplasm around the nucleus. Fragments of endoplasmic reticulum, numerous monosomic ribosomes, and an occasional mitochondrion are the organelles observed. X4,050 and X28,300.
plasm containing a centriole and associated microtubules, profiles of the endoplasmic reticulum and the Golgi apparatus, and numerous polyribosomes (Fig. 6).

**Fine structure of CHO cytoplasts**

Fig. 7 is a thin section of a CHO cytoplast after 3 hr recovery from enucleation. All cytoplasmic organelles are present and except for the missing nucleus, the image approaches that of normal cytoplasm. The centrioles and surrounding centrosphere are always present in the cytoplast, as far as we have been able to determine, and have never been observed in karyoplasts. Fig. 8, which is a higher magnification of part of Fig. 7, shows not only a centriole and associated microtubules but also dark-staining membranous elements reminiscent of the contents of lysosomes. These elements have not been seen in control CHO cells. The Golgi complex and other cytoplasmic organelles of cytoplasts are essentially normal in their morphology. However, after 24 hr the Golgi cisternae are less numerous and abnormal in shape. Dilation also occurs in the cisternae of the granular endoplasmic reticulum. By 48 hr after enucleation, the cytoplasts are generally devoid of elements of either the endoplasmic reticulum or Golgi.

**Fine structure of CHO karyoplasts**

Among the karyoplasts that are present in the pellet after enucleation, there are usually a few whole cells that were in mitosis at the time of centrifugation. These mitotic cells can be removed before the enucleation by prespinning the coverslips in calcium-free medium. The group of karyoplasts in Fig. 9, however, are from a nonsynchronous population of CHO cells that was not prespun and therefore includes one whole cell in mitosis. Fig. 10 depicts a portion of a CHO karyoplast at high magnification. The amount of cytoplasm that comes off with the nucleus varies somewhat, but when distributed more or less evenly around the nucleus comprises a layer not more than 1.0–2.0 μm thick. Ribosomes, mostly monosomic, are abundant; mitochondria and profiles of the endoplasmic reticulum are less so. We have not encountered identifiable Golgi vesicles, centrioles, or microtubules in any of these preparations. Though the karyoplasts appear not to undergo any great changes structurally, they have not been observed to remain intact beyond 72 hr. They seem to have lost the capacity to move about on the substrate.

**Transmission microscopy of whole CHO-cells**

Observations on whole cells and cytoplasts were also made with a JEM 1000 electron microscope operated at 1 MV (Figs. 11 and 12). The cytoplasts (Fig. 12) appear similar to whole cells except for the missing nucleus. In the intact cell (Fig. 11) the mitochondria extend into the peripheral processes, whereas in most cytoplasts examined, few cell processes are observed and the mitochondria appear to aggregate around the cell center (Fig. 12).

**DISCUSSION**

The drug, cytochalasin B, can be used in conjunction with mild centrifugation to separate cells into nuclear (karyoplast) and cytoplasmic (cytoplast) components, and the form and behavior of these components has been carefully observed. Two facts about these components emerge as striking and interesting. One is that the enucleation procedure clearly results in a clean separation of the nucleus from the centrosphere and all microtubules. [Wilson (16) defines the centrosphere as the centrally located, organizational center of the cell and refers to it as a central portion of the cytoplasm that contains centrioles and a dense surrounding zone from which mitochondria and other large inclusions seem to be excluded.] Since the cytoplasts, unlike the karyoplasts, move in a manner comparable to the normal nucleated cell and since they are capable of spreading out on a substrate and adopting a form similar to the original cell, it appears that the enucleation procedure separates the form-determining and motility mechanisms from the nucleus and, moreover, that these mechanisms operate without nuclear participation during the short period of cytoplast viability.

The other observation of interest is that even though endowed with everything usually considered essential for viability, the karyoplasts cannot survive and/or regenerate their lost parts. They have the entire genetic material, intact nuclear envelope, mitochondria, at least fragments of the endoplasmic reticulum, an obvious quantity of ribosomes and a continuous plasma membrane. Why then do they fail to regenerate? Since whole cells exposed to cytochalasin survive the treatment and continue, after removal of the drug, to grow and multiply, it would seem that the cytochalasin treatment is not responsible for the death of the karyoplasts. Furthermore, since they have intact plasma membranes, it is
probable that the exchange of metabolites and metabolic wastes is not at fault. Instead one is led to assume that the organization of cytoplasmic elements relative to the centrosphere is essential to the life of these protoplasts. Possibly the directional channelling of cytoplasmic flow, known to be dependent on microtubules (17), is a factor in these phenomena. The failure of these karyoplasts to survive is made the more remarkable by observations on protozoa, particularly amoebae (18, 19) and certain ciliates (20), which describe these cells as capable of remarkable regeneration after losing large parts of their cytoplasm.

The eventual death of the cytoplasts is not so unexpected. Enucleated cells have without exception failed to survive for an extended period, though oocytes have been reported to segment irregularly for a while in the absence of nuclei (21). Goldstein (22, 23) has made observations on enucleated fragments of HeLa cells and has described behavioral characteristics similar to those observed by the cytoplasts studied here. The degree to which his enucleates contained the central apparatus was not recorded but many of the nucleated cell parts in his experiments seemed to survive. The relevance of his observations to those reported here is therefore left in doubt.

In a few respects at least, the behavior of these cytochalasin-generated cytoplasts parallels that shown by enucleated amoebae. This is particularly true for the Golgi complex. According to Flickinger's observations (19), the complex diminishes rapidly in prominence after enucleation of the amoeba as in the CHO cytoplasts and reappears promptly after the nucleus is returned, as does also the capacity to survive. Similarly, the granular endoplasmic reticulum in enucleated amoebae, as in CHO cytoplasts, dilates within the first 24 hr and subsequently degenerates.

Apart from these straight viability phenomena, the two halves display other differences that are quite interesting. The karyoplasts continue a program of abortive blebbing which is similar to that displayed by cells in early G1. In this as in other more normal instances, the meaning of this behavior is far from clear. The image of some of the blebs in thin sections shows them to contain numerous ribosomes and little else (14). Then also the karyoplasts retain a relatively spherical form (fail to spread) as though lacking a mechanism (cytoskeleton) for imposing any anisometry on the "cells". As far as we have been able to determine the karyoplasts are incapable of motion.

The cytoplasts, on the other hand, for a period of 24 hr display most of the behavioral characteristics of intact nucleated cells. Cytoplasts, including the region of the centrosphere, spread out thinly. They thus adopt a shape that reflects the presence of some form-determining mechanism as previously reported (24). Centrioles, microtubules and microfilaments, which are present in the cytoplasts, are not found in the relatively isometric karyoplasts. One finds in this behavior of the cytoplasts added support for the assumption that form-

The karyoplasts must be deemed to be a viable cell, indispensable to the maintenance of a nucleus. This is presumably the reason why the centrosphere plays an indispensable role in the viability of the cell; that they express themselves, in part, through microtubule and microfilament assembly and distribution which are apparently controlled by the central apparatus including centrioles.

If indeed the centrosphere plays a role during development and the associated changes in cell form, one must conclude that the centrosphere contains structural information that is programmed, along with other major components of the zygote, to exert throughout development its influence in response to induction and other factors.

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