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Cell Biology

Autonomous movements of cytoplasmic fragments

(amoeboid motion/cytoplasm/movement control)

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ABSTRACT **Tiny fragments from the cytoplasm of human skin fibroblasts with about 2% of the original cell volume ("microplasts") were prepared by treatment with cytochalasin B, vigorous pipetting, and trypsinization of the attached fragments. They remained alive for 8 hr or longer. Some of the microplasts were able to produce and move filopodia, ruffle, or both; others blebbed continuously. Slow flattening was observed in the larger microplasts. In all cases tested, microplasts avoided contact with other cells or microplasts. The observations suggest that the cytoplasmic matrix and the membranes of animal cells are so constructed as to express locally and autonomously any one of the elementary amoeboid movements listed above. More importantly, whatever types of motile surface projections a microplast expressed, it continued to produce and move them in a stereotypical way as if there were long-lived structural or material determinants for each type. The microplasts were unable to locomote autonomously. Therefore, it is conceivable that directional movement of whole cells may require a supervising mechanism that confers a certain coordination and strategy on its component cytoplasmic bits. Otherwise they would continue to move in stereotypical and autonomous ways without ever displacing themselves, as suggested by the behavior of the microplasts.**

Inasmuch as sarcomeres are the structural units of muscle cell contraction, are there also structural units of amoeboid motion of nonmuscle cells? (In this context, "amoeboid motion" is understood to mean animal cell movements involving complex body deformations, as opposed to longitudinal contraction of muscle cells or the use of cilia and flagella.) It seems logical to begin the search for such units in the smallest cytoplasmic fragments—one may call them "microplasts"—that can still display autonomously the entire spectrum of amoeboid motion.

Important steps toward the investigation of autonomous cytoplasmic movements have already been taken. Allen *et al.* (1) showed that the cytoplasm of the giant amoeba (*Chaos chaos*) can be forced into glass capillaries, where it continues to stream. Kojima (2) demonstrated that enucleated halves of sea urchin eggs can be induced to cleave. Several years ago, Goldman *et al.* (3) showed that enucleated animal cells (cytoplasts) generated and moved surface projections and also locomoted like whole cells. Even smaller motile cytoplasts of leukocytes were produced by Keller and Bessis (4). In response to temperatures of 46°C, the cells segregated into nucleoplasts and cytoplasts, which continued to migrate, to phagocytose, and to accumulate around chemotactic targets.

Cytoplasts can be fragmented further. Goldstein *et al.* (5) used fine glass needles to cut sizable pieces away from human cells and obtained motile fragments. Much smaller motile fragments were described by Shaw and Bray (6) and later by Wessels *et al.* (7), who found that the similarly amputated distal fragments of axons were able to rebuild a ruffling growth cone. The smallest motile portions of cytoplasm known so far are blood platelets (thrombocytes). Allen *et al.* (8) described several motile abilities of these tiny cytoplasmic fragments of megakaryocytes.

This paper is concerned not merely with fragmentation of the cytoplasm, but with the search for the smallest fragments that still express one or another of the entire repertoire of amoeboid motions. Therefore, platelets and amputated growth cones appear of particular interest. However, one may object that platelets should not be considered as fragments but as highly specialized products of megakaryocytes. Furthermore, the autonomous movements of a growth cone may also represent a very special case and may not imply divisibility of cytoplasm in other cells into tiny autonomously moving fragments. After all, the growth cone composes the entire amoeboidly moving cytoplasm of a neuron, which has to operate centimeters or even meters away from the nerve-cell body and thus needs a certain degree of autonomy.

Contrary to this objection, the present paper demonstrates that a similar autonomy is found in fibroblast cytoplasm. It describes a method of preparing very small, live, cytoplasmic fragments from flattened cultured fibroblasts and shows that they express vigorous amoeboid motion. The smallest fragments that were still able to ruffle or to bleb, etc., were estimated to be about 150 μm^3—i.e., less than 2% of the volume of the original cells as judged by their spherical dimensions after trypsinization.

MATERIALS AND METHODS

The human skin fibroblast clones GRC-161 and GRC-166 used in this study were kind gifts of Edward L. Schneider (National Institute on Aging, Bethesda, MD) and James R. Smith (W. Alton-Jones Cell Science Center, Lake Placid, NY). The cells were grown in Dulbecco's modification of Eagle's medium (GIBCO) supplemented with 10% fetal calf serum (Reheis, Phoenix, AZ) in Falcon plastic dishes (Bioquest, Oxnard, CA). After they reached confluence, cytochalasin B (Aldrich) was added to the culture dishes at 5 $\mu g/ml$ for 30 min from stock solutions of 1 mg of cytochalasin B per ml in dimethyl sulfoxide. Subsequently, the culture medium was pipetted vigorously 5–10 times against the bottom of each dish in order to tear off most of the by now arborized cells, leaving behind their cellular attachment areas and whole cells as well. After two washes with normal culture medium, the attachment areas (fragments) were allowed to heal and reflatten for 30 min in normal medium containing 10% calf serum at 37°C (rehealing period). Subsequently, they (together with the remaining whole cells) were trypsinized and replated on 22 × 22 mm glass coverslips or smaller plastic dishes by standard tissue culture techniques. The coverslips with the replated fragments and whole cells were mounted face down on microscope slides, sealed with wax around the edges to form an observation chamber, and used for live fragment observation in a Zeiss photomicroscope II, with an air curtain incubator (Nicholson Precision Instruments,

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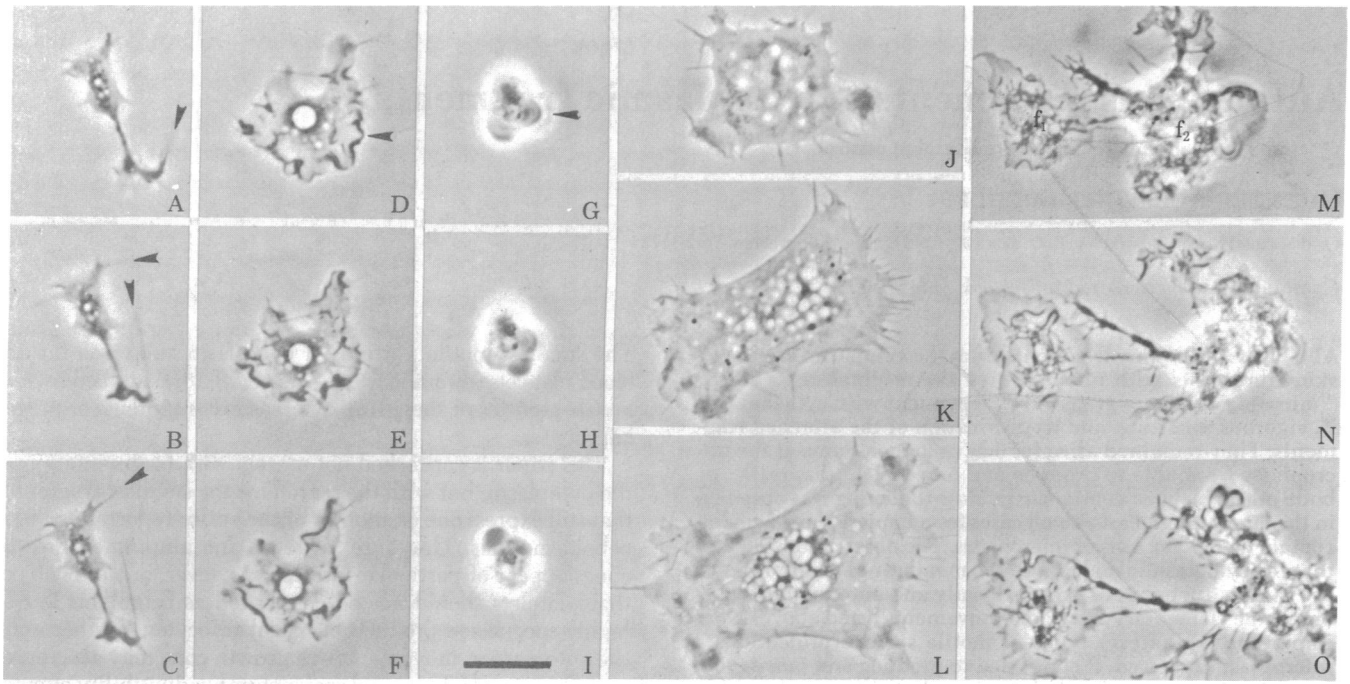

FIG. 1. Various amoeboid movements expressed by replated cytoplasmic fragments. Bar in *I* indicates 10 μm. (*A–C*) Waving of filopodia (see arrowheads). Time interval between *A* and *C* is 15 s. (*D–F*) "Isolated ruffle." Arrowhead points to one of the many ruffling edges. The central white circle is a large pinocytic vesicle. Note also the waving filopodia. Time interval between *D* and *F* is 15 s. (*G–I*) Blebbing fragment. Arrowhead points to one of the blebs. The fragment did not rotate. Times: (*G*) 0, (*H*) 15 s, (*I*) 2 min. (*J–L*) Flattening of a large fragment. Times: (*J*) 0 min, (*K*) 97 min, (*L*) 211 min. (*M–O*) Avoidance reaction between two fragments. Fragment f_1 remained in place while fragment f_2 moved to the right, as determined by marks on the substrate. Fragment f_2 could not retract its tail-like structure in the center of the photographs. Times: (*M*) 0 min, (*N*) 16 min, (*O*) 34 min.

Bethesda, MD) to maintain 37°C. Fragments replated on circular coverslips of 12 mm diameter were examined in an AMR-VTC scanning electron microscope (Advanced Metals Research, Burlington, MA) after being prepared by standard techniques involving critical-point drying. Fragments were prepared for transmission electron microscopy after replating

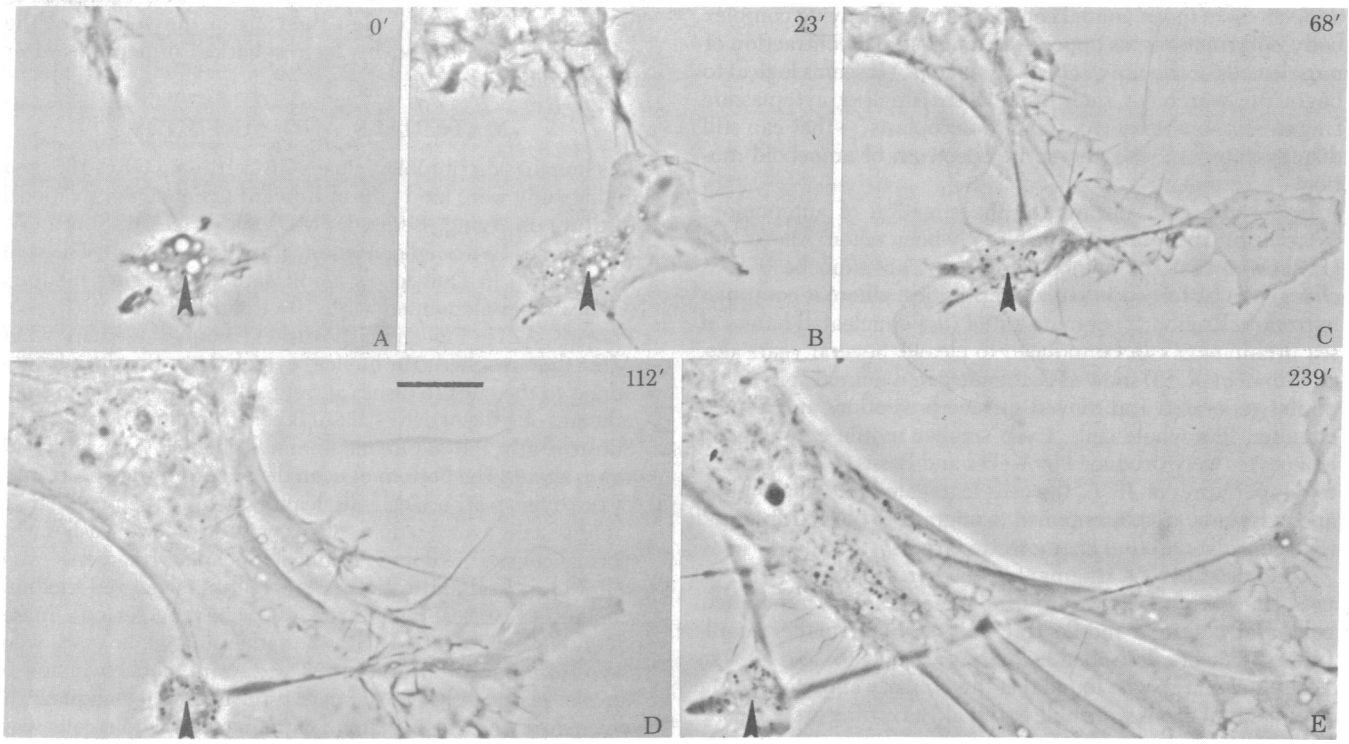

FIG. 2. Avoidance reaction between a replated fragment and the edge of a flattening cell. Bar indicates 10 μm. Numbers on the panels indicate the times of observation, in min. The part of the fragment indicated by the arrowhead remained at the same spot as judged by substrate marks. After the approaching cell made contact with the fragment, a ruffling lamellipodium of the fragment formed and moved to the right while the further advancing cell edge underlapped the cytoplasmic bridge between the two portions of the fragment.

on plastic dishes and fixation 1 hr later. The embedded preparations were sectioned parallel to the substrate and examined in a Phillips 201 electron microscope.

RESULTS

***In Situ* Fragments.** After the 30-min incubation in normal culture medium, the attached pieces left behind by the arborized and squirted off cells rehealed to form spindly or triangular fragments (*in situ* fragments). As determined by transmission electron microscopy of five *in situ* fragments, sectioned parallel to the substrate, they contained prominent and straight microfilament bundles, usually lining the fragment's edges. There were also numerous microtubules, bundles of intermediate filaments, vesicles, mitochondria, and ribosomes. The *in situ* fragments displayed little movement. When they were exposed to a second treatment with cytochalasin B at 5 μg/ml for 30 min, their thinner parts retracted into thickening knobs. Incubation of *in situ* fragments with Colcemid at 1 μg/ml for 1 hr resulted in increased mobility of cytoplasmic vesicles and mitochondria, and the disappearance of microtubules, as determined by transmission electron microscopy of eight fragments.

Replated Fragments. The *in situ* fragments responded to trypsinization and replating on new substrates with dramatically increased amoeboid movements. For 8 hr or longer after separation from the original cell, the replated fragments generated waving filopodia (Fig. 1 *A–C*), ruffling lamellipodia (Fig. 1 *D–F*), or blebs (Fig. 1 *G–I*), indistinguishable from those of whole cells. Larger fragments flattened like whole cells (Fig. 1 *J–L*). In no case, however, did the movements of the replated fragments result in locomotion, although displacement of parts of fragments could be observed after contact with an adjacent fragment (Fig. 1 *M–O*) or cell (Fig. 2). Ruffling parts of fragments were seen to move away from their initial contact areas on the substrate while leaving attached, nonruffling parts behind. Fig. 2 shows a particularly dramatic case of "contact-avoidance" of a fragment that lengthened itself considerably in response to an approaching cellular edge. In contrast to whole cells, the contact-avoiding fragments were apparently unable to pull their rear portions permanently toward their fronts.

Fig. 3 shows scanning electron micrographs of several replated fragments and, for size comparison, of a reflattening whole cell as well (Fig. 3*A*; for another size comparison between cells and fragments see Fig. 2). The filopodia (f in Fig. 3*B*), ruffling lamellipodia (rl in Fig. 3*D*), and blebs (bl in Fig. 3*C*) produced by the fragments appeared indistinguishable from those of whole cells.

Transmission electron microscopy of 28 fragments, fixed 1 hr after replating and sectioned parallel to the substrate, showed a rather uniform ultrastructure of microplasts. Usually there was a core of organelles intermingled with intermediate filaments (Fig. 4*E*). Microtubules often crossed the core and looped around the perimeter (Fig. 4*B*). Microfilaments in sections higher than the most ventral sections circled the core in a dense ring (Fig. 4 *A* and *F*). Outside this ring there was usually a ribosome-rich meshwork type of cytoplasm. This organization was seen in blebbing and in ruffling microplasts alike, as will be described in more detail elsewhere. Prominent densities could be seen along the curving microfilaments in several fragments (Fig. 4*F*). Similar striations have been observed in whole cells by transmission electron microscopy after tannic acid staining (9). Two fragments showed microfilament arrangements that converged toward vertices (Fig. 4 *C* and *D*), similar to the polygonal networks observed in whole cells (10, 11). The microtubules that looped around the perimeter of the microplasts (Fig. 4*B*) occasionally seemed to terminate about 800 Å away from the plasma membrane. It remains to be determined what structures, if any, nucleated these microtubules in the microplasts, which do not have centrioles. Some microplasts showed all three types of filaments. In others, not all types could be seen with certainty, presumably because some sections were obscured by grid bars.

It is interesting to note that ruffling microplasts continued to ruffle for hours at the same rate. They never changed into blebbing microplasts by themselves, or vice versa. Preliminary observations with microplasts prepared from various other cell

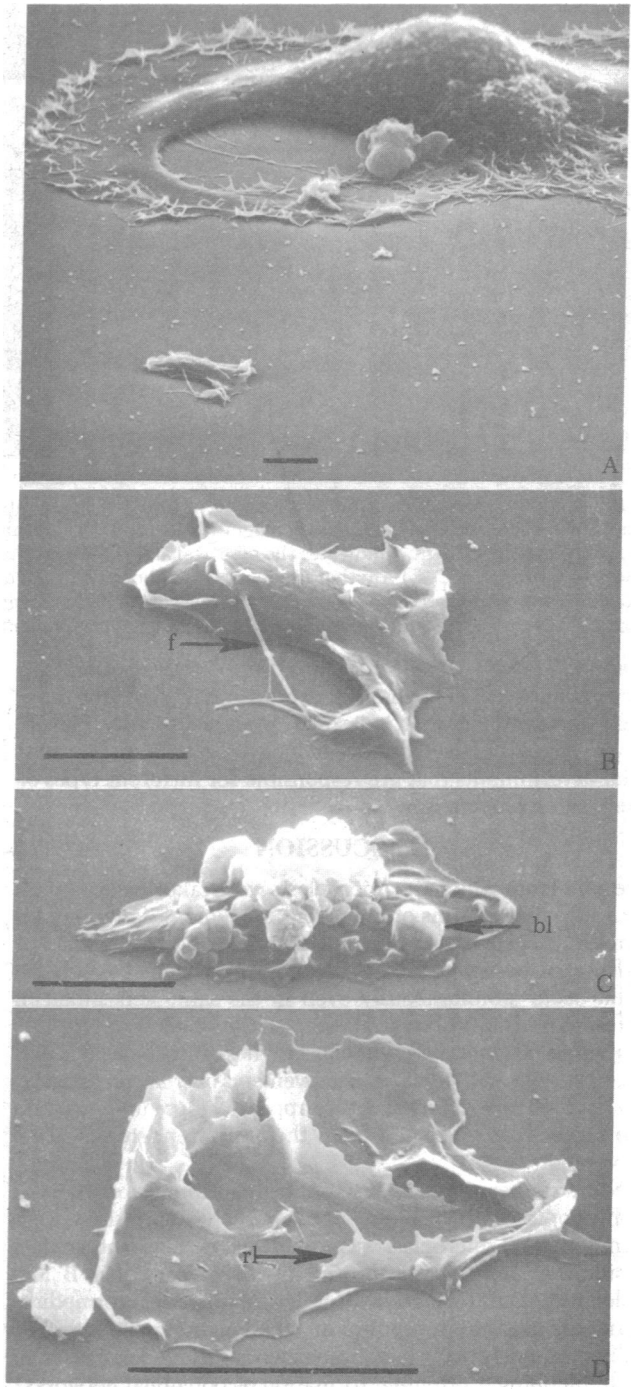

FIG. 3. Scanning electron micrographs of various replated fragments. All bars indicate 5 μm. (*A*) A fragment together with a flattening human skin fibroblast. (*B*) The same fragment in higher magnification; f indicates one of the filopodia. (*C*) Another fragment showing blebs (bl). (*D*) An isolated ruffle; rl points to one of the many ruffling lamellipodia of the fragment.

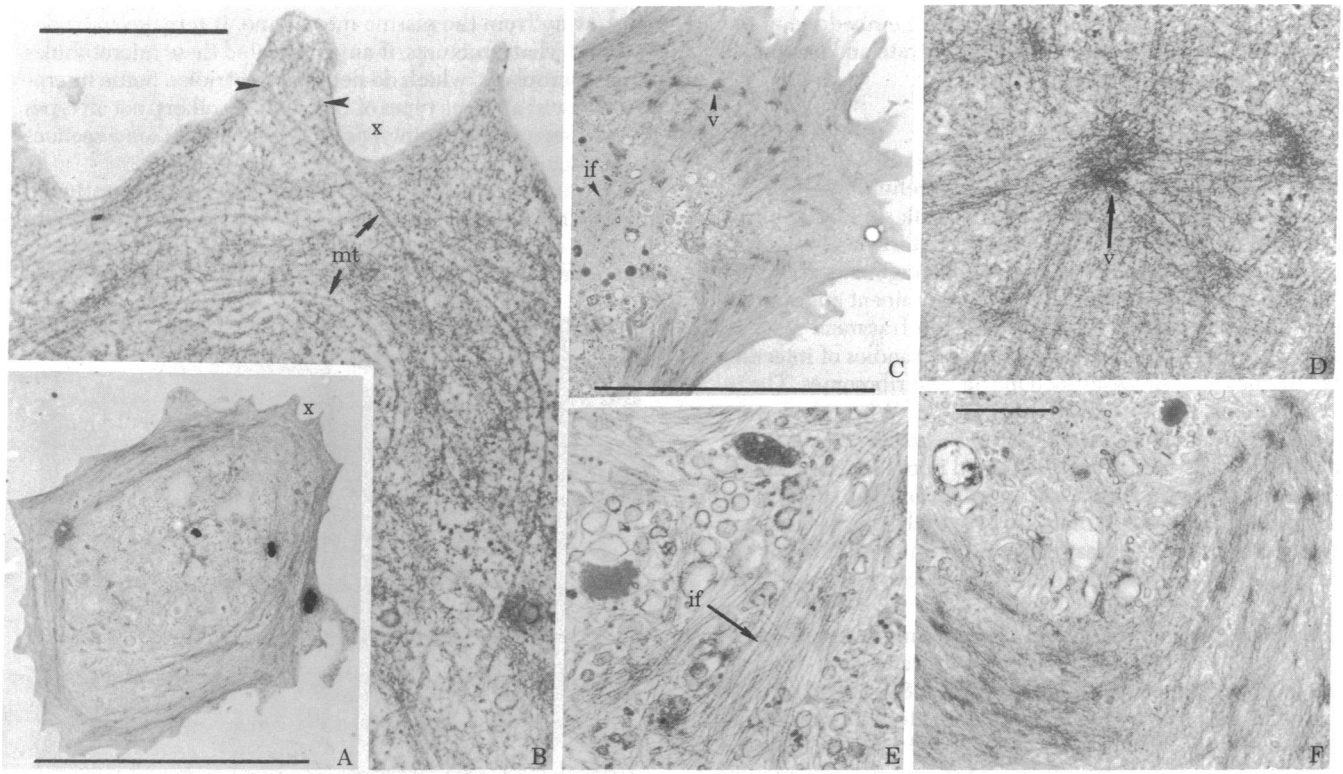

FIG. 4. Transmission electron micrographs of replated fragments sectioned parallel to the substrate. Bars indicate 10 μm (*A* and *C*) and 1 μm (*B* and *F*); *D* and *E* are at the same magnification as *B*. (*A*) Circumferential microfilament bundles in a fragment. (*B*) Looping microtubules (mt) in the same fragment. x indicates the identical areas in *A* and *B*. Arrowheads indicate points very near the membrane where microtubules appear to terminate. (*C–E*) Another fragment, with about twice the diameter of that shown in *A*. It displays polygonal network organizations. v points to one of the vertices. *D* shows the same vertex in higher magnification. if points to bundles of intermediate filaments, shown in higher magnification in *E*. (*F*) Psuedoperiodic densities along microfilament bundles near the periphery of another fragment.

types (3T3, Py3T3, neuroblastoma) support the notion that microplasts, if left to themselves, express continuously the same forms of movement, each in its own characteristic way. Hence, they may point to the existence of long-lived structural or material determinants that are responsible for different types of cytoplasmic movements.

DISCUSSION

The cells from which the microplasts were taken expressed all the well-known forms of amoeboid movement, including locomotion. Particularly during flattening, the movements of ruffling, blebbing, waving of filopodia, and contact avoidance could occur simultaneously even in the same cell, albeit in different parts of its body. In contrast, the smallest microplasts seemed to fall into different classes whose members repeated a certain type and sequence of movements over and over again. Interestingly, their ultrastructure appeared more uniform than one might have expected from their different stereotypical movements.

Numerous expressions of whole cell motility were observed in microplasts. Regardless, whether these expressions were common to all microplasts (e.g., substrate attachment, contact avoidance, morphological response to cytochalasin B and Colcemid, and formation of microfilaments, intermediate filaments, and microtubules) or whether they characterized individual microplasts (e.g., movement of filopodia, ruffles, or both, blebbing, flattening, formation of polygonal networks), they required no more than 100–200 μm^3 of cytoplasm surrounded by a portion of plasma membrane. Apparently, the design of the cytoplasmic matrix and cell surface is complex enough to sustain this variety of expressions of cell motility locally and autonomously.

Consider now the possibility of fusing single microplasts to reconstitute a whole cell's cytoplast. Obviously, it would be necessary to coordinate their autonomous actions, or else paralysis and tremor of the cytoplast would result. Therefore, one has to postulate that the various parts of a whole cell's cytoplast are attuned to one another by some unknown communication mechanism. Still, this postulate would not suffice to explain locomotion of cytoplasts. A certain strategy for the shape changes of each coordinated microplast seems necessary before the cytoplast as a whole can extend and retract parts of its body in the proper time sequence required for locomotion. Therefore, it seems that an additional supervising mechanism must exist in locomoting cytoplasts and, therefore, in intact cells. It is not known at the present time how the postulated supervising mechanism might operate.

As much as the autonomy of the microplast movements points to the existence of an unknown communication mechanism between the different parts of a locomoting cytoplast, their stereotypical movements long after separation from the cell point to long-lived determinants of one or another type of movement. At the present time it is not known what these determinants are. Conceivably, they are cellular compounds that were accidentally trapped inside the microplasts during the preparation procedure. Alternatively, they may be fortuitous physical properties, such as the volume or surface of a microplast, its firmness of attachment to the substrate, the relative amounts of microfilaments, microtubules, etc., or any combination of these. More intriguingly, the microplasts may hint at the existence of a special class of compounds which restructure the cytoplasm, the surface, or both in such a way that it becomes locked into the expression of one or another type of movement. Experiments such as the fusion between, e.g., blebbing and ruffling microplasts of different or equal sizes may reveal

whether the postulated determinants are related to composition, organization, or size of microplasts.

The existence of microplasts encourages the belief that there are units of amoeboid motion and that they may be approached experimentally by preparation of microplasts. It seems conceivable now that the complexity of tissue cell movements may be understood as the result of many such autonomous units communicating and cooperating under the influence of a supervising mechanism.

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