Cytostructural Dynamics of Spreading and Translocating Cells

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ABSTRACT Cytostructural changes during fibroblast spreading and translocation and during the transition between the two states have been studied in living cells and in the same cells after fixation and immunofluorescent staining. In time-lapse sequences we observe that birefringent arcs, sometimes circles, concentric with the cell perimeter, form near the periphery of a spreading cell, or that arcs form near the leading edge of a locomoting cell. The arcs move toward the nucleus, where they disappear. In spreading cells, radial stress fibers extend from the region of the cell nucleus to the periphery. The arcs or circles and the stress fibers are visualized in the same cells after fixation and staining with fluorescein-conjugated antiactin antibodies. Stained images of spreading cells show the arcs and stress fibers in the same plane of focus. At points of intersection with arcs, stress fibers are bent toward the substrate on which the cell is moving.

During a transitional stage between spreading and translocation the cytostructure undergoes reproducible changes. Arcs and circles cease to form. The radial stress fibers elongate, spiral around the nucleus, and move to the periphery as a band of filaments.

We interpret the moving arcs as condensations of a microfilament network that move toward the nucleus as compressional waves. As elements of the net are brought close together by the compressional wave, contraction may occur and facilitate the condensations.

The way in which the cytostructure participates in and accommodates to the motility of tissue cells has become a subject of broadening interest. Recognizing that motility is a dynamic phenomenon, a number of recent studies have related the motile state of cells at the time of fixation to cytostructural details revealed by electron microscopy or immunofluorescent staining. For example, the low density or absence of stress fibers (1–4), the absence of membrane structures specialized for adhesion (2) and the absence of a requirement for tails (3) have been reported as characteristics of translocating cells.

Optical systems (5, 6) that permit resolution of many features of the cytostructures of living tissue cells in culture have made it possible to study dynamic changes at high resolution. However, not until recently has a relatively regular and quite invariant pattern of cytostructural changes in living tissue cells been correlated with cell spreading and translocation (7). It is the purpose of this paper to detail more fully the pattern of changes described in our preliminary report, and to suggest how elements of the cytostructure might interact to produce coordinated cell movement.

We were not the first to observe moving arcs in translocating cells. They have been observed by others, but neither their composition nor their significance have been dealt with. Heath and Dunn (8) cite the unpublished observations of Albert Harris and G. A. Dunn: in time-lapse films of translocating chick heart fibroblasts viewed with phase-contrast optics, Harris and Dunn saw phase-dense lines move from the cell periphery to the nucleus. Recently, Heath (9) reported the movement of arcs that he locates close to the dorsal surface of translocating cells. Similar structures have been observed in thin sections of spreading epithelial cells (10, 11).

In this paper we consider the composition of moving arcs and their relationship to other cytostructural elements that participate dynamically in cell spreading, cell translocation, and the transition between the two states.

MATERIALS AND METHODS

Cell Culture and Filming Preparation

Human lung fibroblasts (IMR 90) were cultured in McCoy's 5A medium supplemented with 20% fetal bovine serum (McCoy's/20). Cells were taken from cultures just before they reached confluency. A culture was first washed with Ca^{++}/Mg^{++}-free Puck's B solution and then trypsinized (trypsin-EDTA 1 from Flow Laboratories, Inc., Rockville, M) for 1–5 min at 37°C depending on the age of the culture. Cells released from the substrate were suspended in McCoy's/20

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and plated onto strain-free No. 1½, 24.5-mm circular cover slips. A cover slip, with cells, was incubated for 45 min at 37°C, after which it was assembled into a Dvorak-Stottler controlled environment chamber (Precision Instruments, Bethesda, MD), kept at 37°C throughout filming. Since an upright microscope was used, the cover slip bearing the cells made up the top element of the chamber. The chamber was positioned on a revolving stage of a Zeiss Universal microscope equipped with either phase-contrast, Nomarski-interference, or Nikon-rectified optics for polarization microscopy. We used a compensator made with a split mica sheet of 0.00006 in. thickness positioned between stain-free covers. An Arriflex 16-mm movie camera loaded with Kodak technical pan 2415 film was used to record events during spreading or translocation at a filming rate of 4 exposures/min with polarization or Nomarski optics, or 2 exposures/s with phase-contrast optics. An Arriflex capping shutter below the condenser was closed between exposures.

Time-lapse videotape recordings of spreading or translocating cells were made with a DAGE MTI Nuvicon TV camera interfaced to a Sony TVO 9000 time-lapse tape recorder. A Colorado Video grey scale expander (Colorado Video, Boulder, CO) was incorporated into the recording circuit for image enhancement. The time compression ratio used was 1:12. Taped images were displayed on an Electrohome black and white TV monitor. Pictures were taken from the monitor with a Nikon 35-mm camera at an exposure of 1 s. To remove the TV scan lines on the negative from the final print, the image from the enlarger was slightly defocused.

Antigen Preparation: Extraction Procedure for Actin

A muscle acetone powder was prepared from chicken gizzards according to the method of Kielley and Harrington (12) and extracted according to methods described by Spudich and Watt (13). Polymerization-depolymerization was repeated according to Eisenberg and Kielley (14), and the actin solution was stored frozen.

Antibody Preparation: Injection Schedule

Female New Zealand white rabbits, ages 6–12 wk, were injected each mo with 250 μg of the actin antigen, which had first been made 2% in SDS, as follows: the first month, an intramuscular injection of the antigen in Freund's complete adjuvant was given. The second month, subcutaneous injections of antigen in saline were given in the dorsal surface of all four feet. Subsequent injections in Freund's incomplete adjuvant were given in the back subcutaneously. 2 wk after each injection, the rabbits were bled from the ear and the serum was stored frozen until used.

Antibody Purification

The extracted actin was loaded onto a column containing DNase I linked to Sepharose 4B. The DNase-Sepharose was prepared by the CNBr activation method according to a procedure described by Kato and Anfinson (15); actin was eluted from the column according to the method of Lazarides and Lindberg (16).

The purified actin was coupled to CNBr-activated Sepharose and loaded into a column. Rabbit serum containing antibodies to actin was loaded onto the actin–Sepharose column. The actin-specific antibodies were eluted from the column using 0.2 M glycine-HCl at pH 2.3.

A preparation of monoclonal antibodies prepared against actin (a generous gift from Dr. James Lessard, Children's Hospital Research Foundation, Cincinnati, OH) was also used.

Immunofluorescence: Cell Preparation and Microscopy

Cells were fixed in 3.7% formaldehyde in PBS at 37°C while they were being filmed. The area under observation was then marked and the cover slip was removed from the chamber and placed cell side up into a petri dish containing the fixation solution. The cells were then acetone-postfixed and stained sequentially either with rabbit antiactin and FITC goat anti-rabbit (Miles Laboratories, Elkhart, IN) or with monoclonal mouse antiactin and FITC rabbit anti-mouse antibodies (Miles Laboratories).

Fluorescein-stained cells were viewed on a Zeiss standard microscope equipped with a Zeiss IV F1 vertical illuminator containing a 485-nm excitation filter and a 520-nm barrier filter. Photographs were taken with a Nikon M-35-5 camera body mounted on a Nikon PFM microflex, using Plus-X film.

RESULTS

Arc and Circle Formation and Propagation in Spreading Cells: Polarized Light Microscopy

Human lung fibroblasts plated on a glass or plastic substrate assume a circular or polygonal shape as they spread over a flat surface. Between 1 and 2 h after plating, cells were viewed from their substratum side in polarized light and seemed to display two dominant types of birefringent structures. The first type consists of radial lines arranged like the spokes of a wheel (Fig. 1 *a*). Usually, the radial spokes terminate just before the advancing cell border. The second type of structure consists of arcs and/or circle arranged parallel to the outer margin of the cell and consequently perpendicular to the radial spokes (Fig. 1 *a*). The arcs or circles form at the periphery of the cells in an area where the spokes terminate, and then move toward the nucleus at a mean rate of 2–3 μm/min. Often a circle of birefringence will form when the ends of separate arcs, moving toward the nucleus, merge (Fig. 1 *a*).

A succession of arcs may form in the same region and follow one another to the perinuclear zone (Fig. 2). If the area of the circular cell has increased in the interim, the radius of the newly formed arc or circle also increases. The process of arc and circle formation continues while the cell is spreading. The rate of arc and circle movement and the rate of cell spreading are dissimilar. The arcs move at a rate twice that of the rate of increase of cell diameter measured during a given period. In some instances arc formation is clearly preceded by extension of the cell at its periphery. Time-lapse films reveal continuous local distensions and recoils of limited segments of the plasma membrane that occur at a rate much more rapid than that of arc movement.

Arc and Circle Formation in Spreading Cells: Indirect Immunofluorescence

The structures observed with polarized light microscopy also may be visualized in cells processed for indirect immunofluorescence using antibodies against actin (Fig. 1 *b*). With minor exceptions there is a remarkably high degree of correspondence between what is observed in the living cell as birefringent structure and what is revealed after staining with antiactin antibody (Figs. 1 *a* and 1 *b*). A thin bright band demarcating the plasma membrane appears in the fluorescent image when the edge of the cell is hardly visible in polarized light. Some areas of diffuse birefringence can be seen to be filled with stained microfilaments after antiactin staining. Although filaments are numerous everywhere in the cell, they appear more concentrated at the cell periphery and where the radial spokes terminate.

The radial spokes that appear to terminate near or at the plasma membrane adjacent to the glass substrate do not lie parallel to the ventral plasma membrane, but angle toward the dorsal cell surface (as shown schematically in Fig. 9, in which a cell is oriented with its ventral or attached surface up). This is apparent when a spreading cell "hanging" from a cover glass is viewed from above at different planes of focus between its dorsal and ventral membranes (Fig. 3 *a*, *b*). In Fig. 3 *a* the focus is 2 μm below the ventral membrane. Note that the middle section of each spoke is in focus, whereas the perinuclear ends in particular are not. An antiactin-stained ring "C" can be seen out of focus in the central part of the cell away from the ventral surface (Fig. 3 *a*). As the focus is moved 3–4 μm away from the

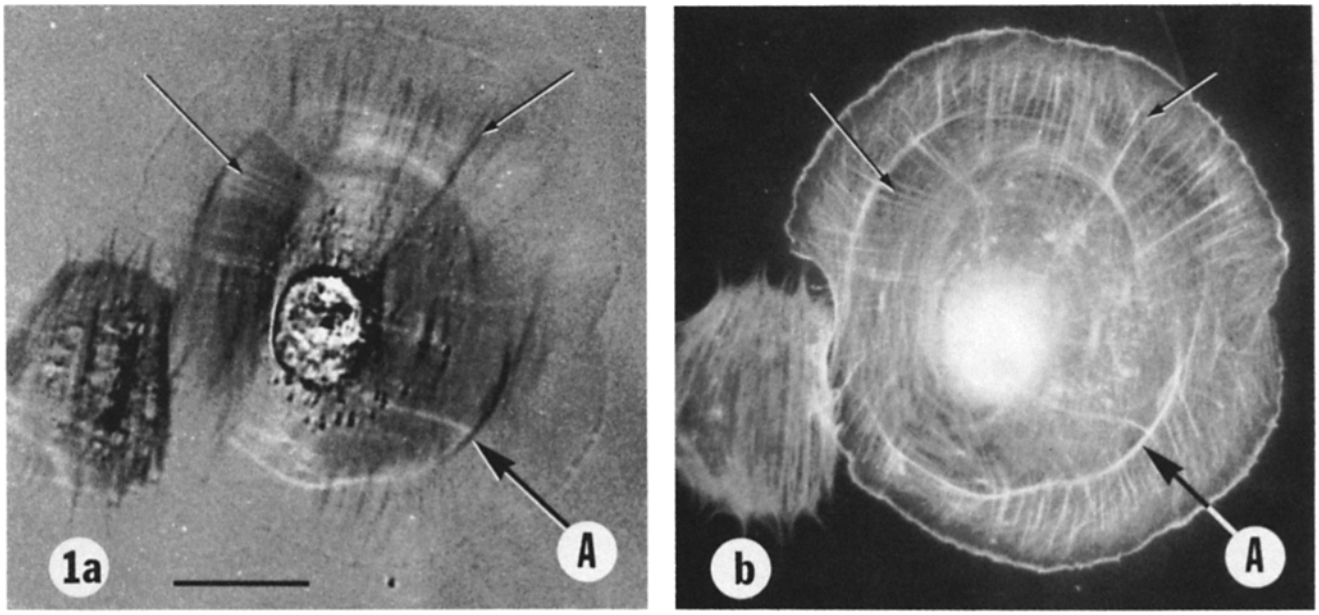


FIGURE 1 (*a*) A spreading cell is viewed with polarization optics. The two predominating structures are radial stress fibers (small arrows) of contrasting birefringence and arcs (*A*) or circles of contrasting birefringence (depending on the quadrant) concentric with the cell periphery. A nearly complete circle has been formed from merged arcs of birefringence. (*b*) The same cell as in *a* fixed with 3.7% formaldehyde in PBS and stained indirectly with fluorescent conjugated antibodies against actin. The stress fibers appear to terminate in a dense filamentous mesh. Note the bend in the stress fibers where they intersect the arcs (small arrows). A thin band of fluorescence can be seen along the entire peripheral membrane. Bar, 10 μm. × 1,800.

ventral surface, which is uppermost, to the focal plane of the ring (Fig. 3*b*), the ends of some radial spokes terminating in the perinuclear region become resolved. Where the spokes are intersected by the ring (Fig. 3*b*), they are bent toward the ventral or substrate cell surface that is above the plane of the photograph; this bend leads us to suggest an association of the spokes with the rings. If the region of the bend is kept in focus (Fig. 3*b*) all the spoke ends at the periphery of the cell (or near the ventral surface) and a few in the perinuclear zone (near the dorsal surface) are out of focus. Optical sectioning of antibody-stained cells shows that the most recently formed arc or circle in cells with more than one arc or circle is closer to the ventral substrate than an arc that is nearer to the nucleus.

The Transition from the Spreading to the Translocating State

POLARIZED LIGHT MICROSCOPY: Late in spreading, formation of the traveling birefringent arcs and circles ceases and the cytostrucure as a whole undergoes a series of dynamic changes. These are shown in photographs from a time-lapse film (Fig. 4). The nucleus and other visible cytoplasmic inclusions begin to rotate. At first, the birefringent radial spokes appear to remain anchored at both ends. The ends associated with the peripheral substrate membrane remain stationary, whereas the ends terminating in the nuclear region move with the rotating nucleus. By measuring spoke length in sequential frames on a Nucmonics tablet, we have determined that they elongate ~30%. As they elongate, the spokes develop a spiral configuration. We postulate that this elongation of the spokes within the limited area of the cell cytoplasm is responsible for the nuclear rotation. The spokes terminate short of the peripheral membrane (Fig. 4*b* and *c*), and between their ends and the limiting edge of the cell lies a circumferential fringe which does not rotate. The fringe persists until shortly before the cell begins to translocate. As nuclear rotation continues, the radial arms distal to the nucleus are brought adjacent to one another. The outline of a circle of radius smaller than the cell begins to take shape. The radial arms no longer appear anchored in the perinuclear region and begin to move from the nucleus toward the periphery, forming a circle of filaments. The cell appears devoid of birefringent structures except for the circumferential band of filaments along the periphery which undergoes continuous change, with bands of birefringence appearing and disappearing (Fig. 4*d*). Unlike the traveling arcs of birefringence, these changes are confined to the cell periphery. Protrusions of the cell membrane begin along one segment of the perimeter, and arcs can be seen forming once again behind the protruding edge (Fig. 4*e–h*). The arcs travel back toward the nucleus in a manner similar to that of those described above. As the membrane protrudes, cytoplasmic particles in the nonbirefringent perinuclear zone can be seen to travel at a high velocity on both sides of the nucleus. One group travels clockwise around the nucleus while the other travels counter-clockwise, both moving toward the developing lamellipodium.

Immunofluorescence Observations

All birefringent structures seen with the polarization microscope also appear in the immunofluorescence images after antiactin staining. The cell pictured in Fig. 5*a* was at the same stage of rotation of cytostructure as the one shown in Fig. 4*b* when fixed. Similarly, the cell pictured in Fig. 5*b* was at a stage comparable to that of the cell seen in Fig. 4*e*. The fibers seen in a cell beginning to translocate are much thicker than those seen in a spreading cell. Prominent dark patches appear between bundles of fibers (Fig. 5*b*), suggesting areas devoid of actin or actin-containing structures. As a result of the cytostructural reorganization, the radial spokes are transposed to become circumferential fibers located at the cell periphery (Fig. 5*b*).

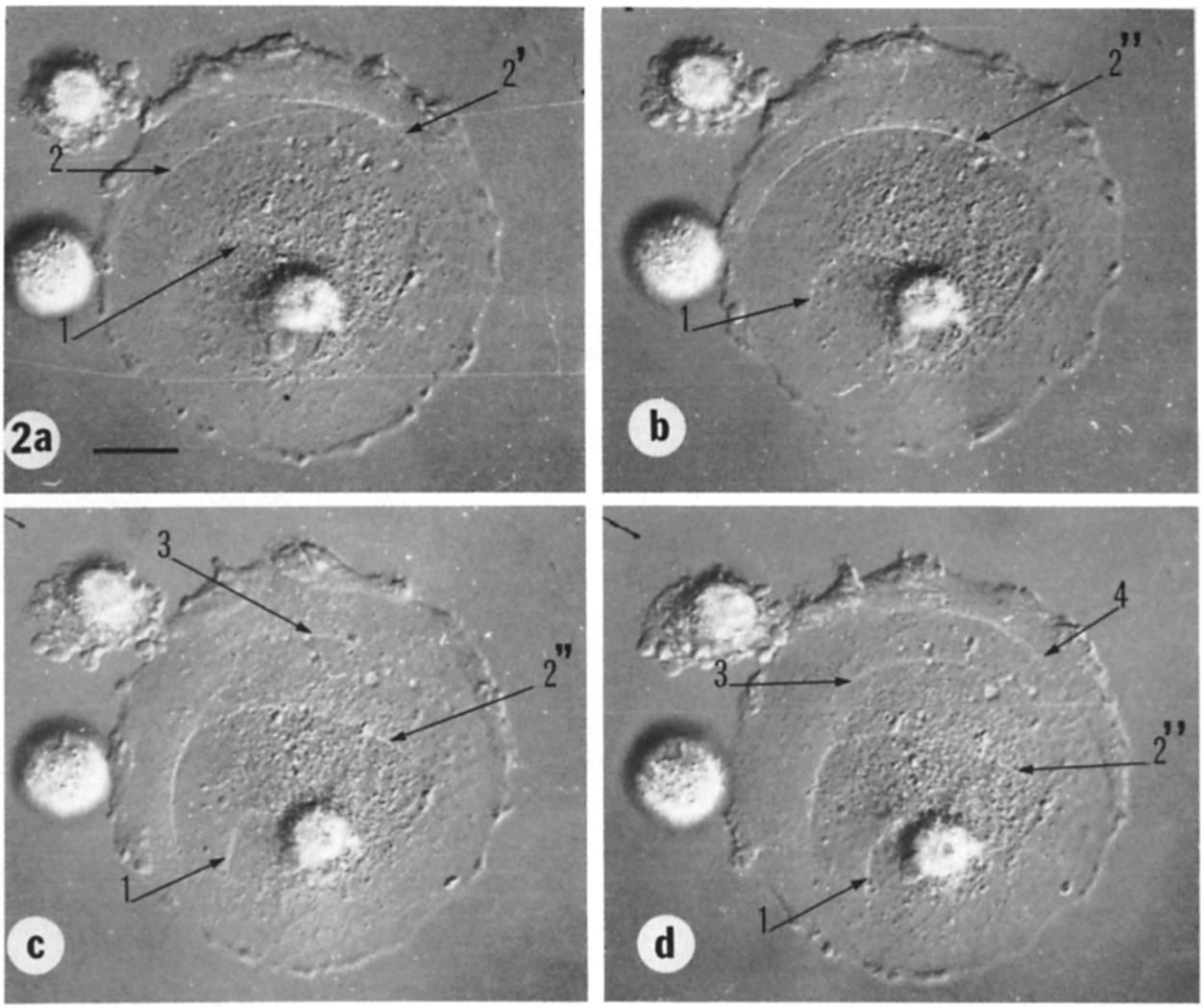


FIGURE 2 Nomarski differential interference-contrast images of arcs continuously forming along one edge of the cell which then move toward the nucleus. (*a*) Two arcs labeled *2* and *2'* in *a* have moved toward the nucleus and merged, forming one large arc 2" (*b*) while arc *1* has moved little. (*c*) A third arc labeled *3* has begun to form at the periphery behind arc *2"*. Both arcs *2"* and *1* have moved toward the nucleus. (*d*) An arc labeled *4* has formed and arcs *3*, *2"* and *1* have moved closer to the nucleus. Bar, 10 μm. × 1,200.

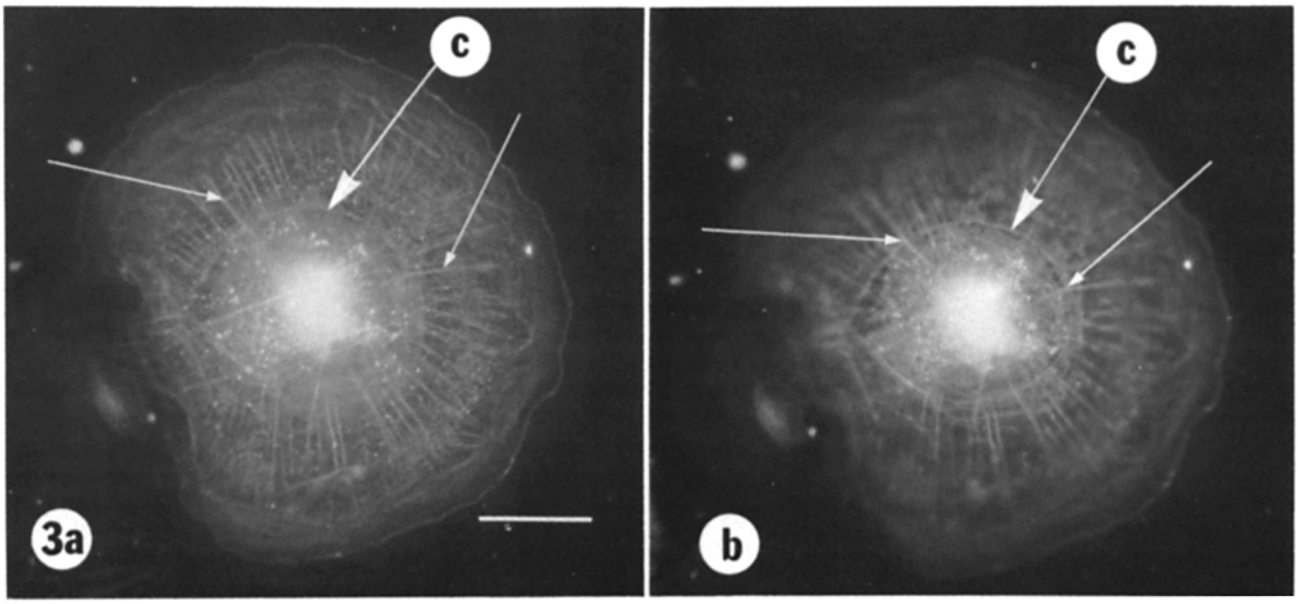


FIGURE 3 Indirect immunofluorescent images of a spreading cell stained with rabbit antibody to actin. The plane of focus in *a* is below the ventral or substrate associated membrane, while in *b* it is closer to the nucleus. In *a* the central portions of the radial stress fibers are in focus (small arrows). The circle of birefringence (*c*) that has moved from the periphery to the immediate perinuclear zone, as seen in the time-lapse film, is out of focus in *a* but in sharp focus in *b*. The stress fibers that the circle intersects appear to be bent upward (small arrows) because the segments previously in focus now are blurred. Bar, 10 μm. × 1,600.

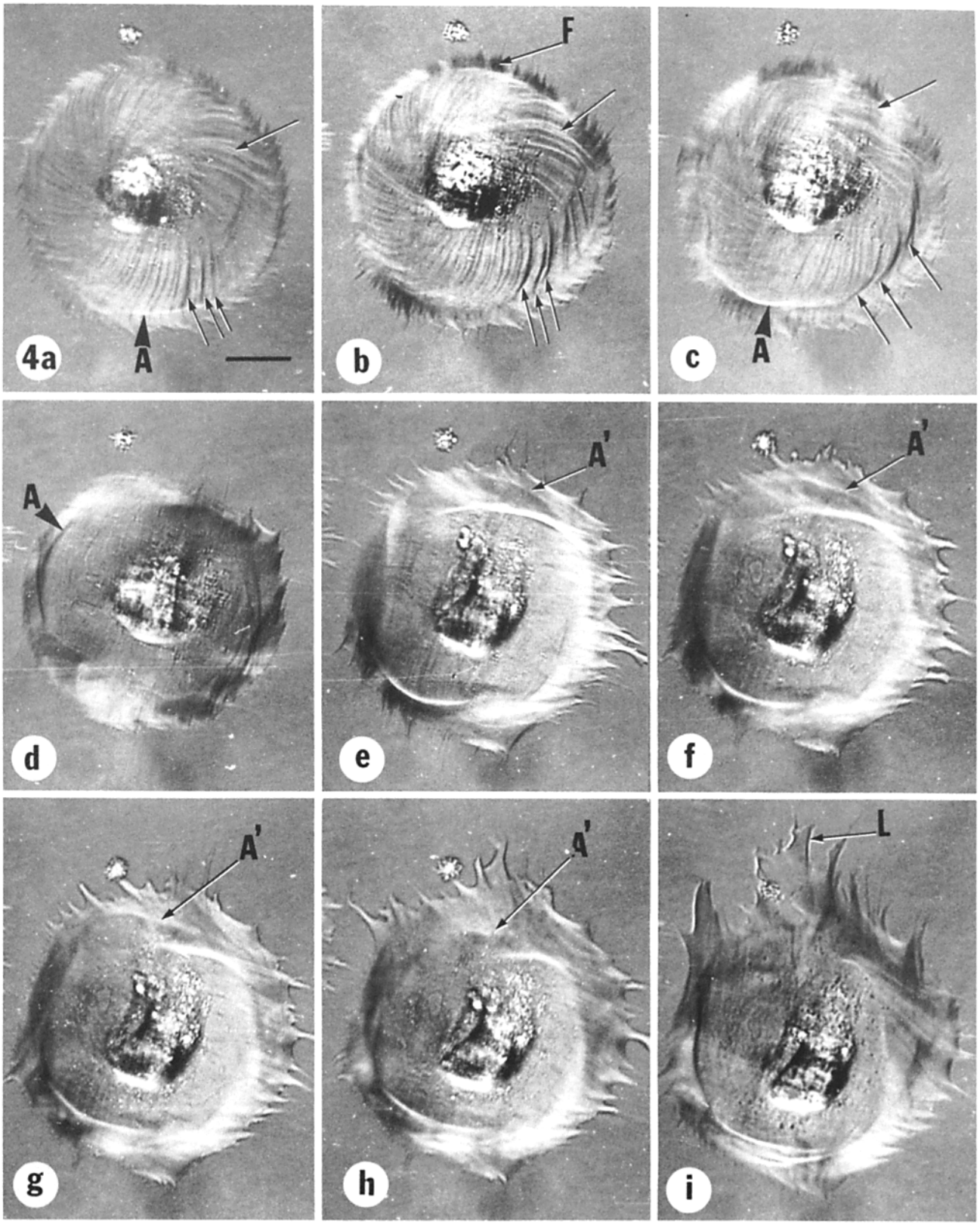


FIGURE 4 Transitional stage during which the cytostructure of a fully spread cell is reorganized prior to locomotion. The radial stress fibers (Fig. 1) begin to bend with rotation of the nucleus. In this sequence they rotate counterclockwise and then move away from the nucleus to form a peripheral band of birefringence. Two groups of stress fibers (small arrows) are labeled to follow their movements. Birefringent arcs (*A*) and circles appear and disappear along the periphery but do not move; examine *a*, *c*, and *d* for example. Note the fringe (*F*) that extends from the peripheral band of birefringent fibers to the cell border. The perinuclear area in *d* appears devoid of birefringence once all stress fibers have moved to the periphery. Arcs (*A'*) that do not move begin to appear at the edge coincident with a forming lamellipodium (*L*), *e–i*. The time interval between *a* and *i* is 3 *h* 20 min. Bar, 10 μm. × 1,250.

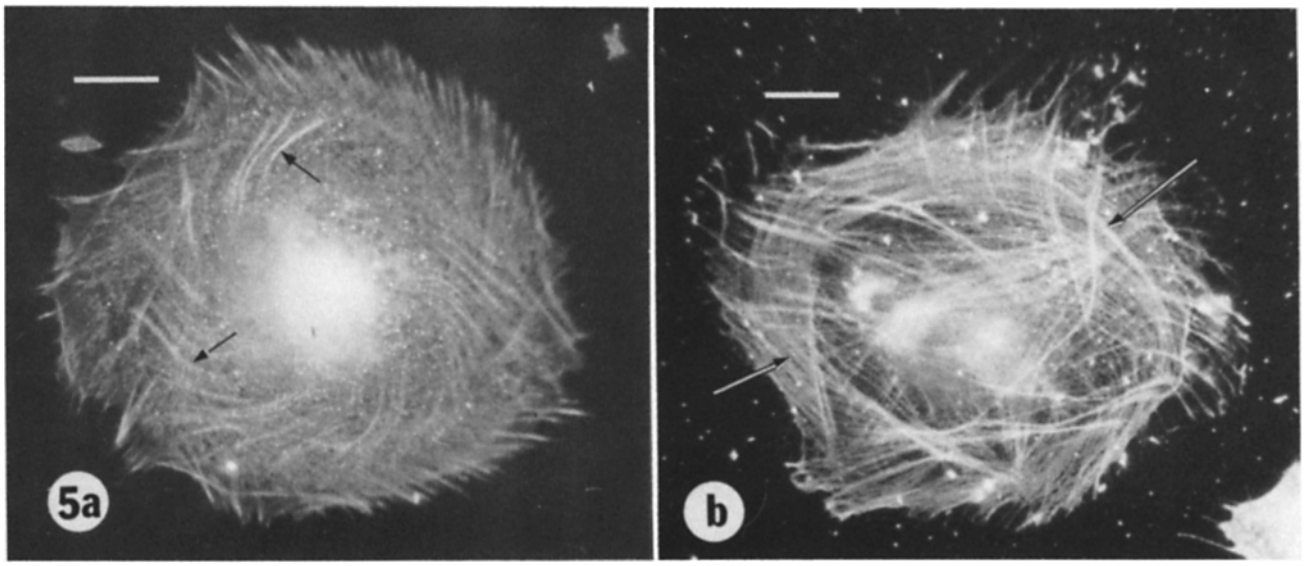


FIGURE 5 (*a*) Indirect immunofluorescent image of a cell stained with antiactin antibody at a stage of transition similar to that in Fig. 4 *b* or 4 *c*. Rotation of nucleus and stress fibers (arrows) was clockwise in this cell. (*b*) Indirect immunofluorescent image of a cell stained with antiactin antibody at a stage similar to that of the cell in Fig. 4 *d*. Note that the distribution of filaments (arrows) is largely in a circumferential band. Portions of the perinuclear area appear black suggesting areas devoid of actin containing structures. (*a*) Bar, 10 μm; × 1,150. (*b*) Bar, 7.3 μm; × 1,350.

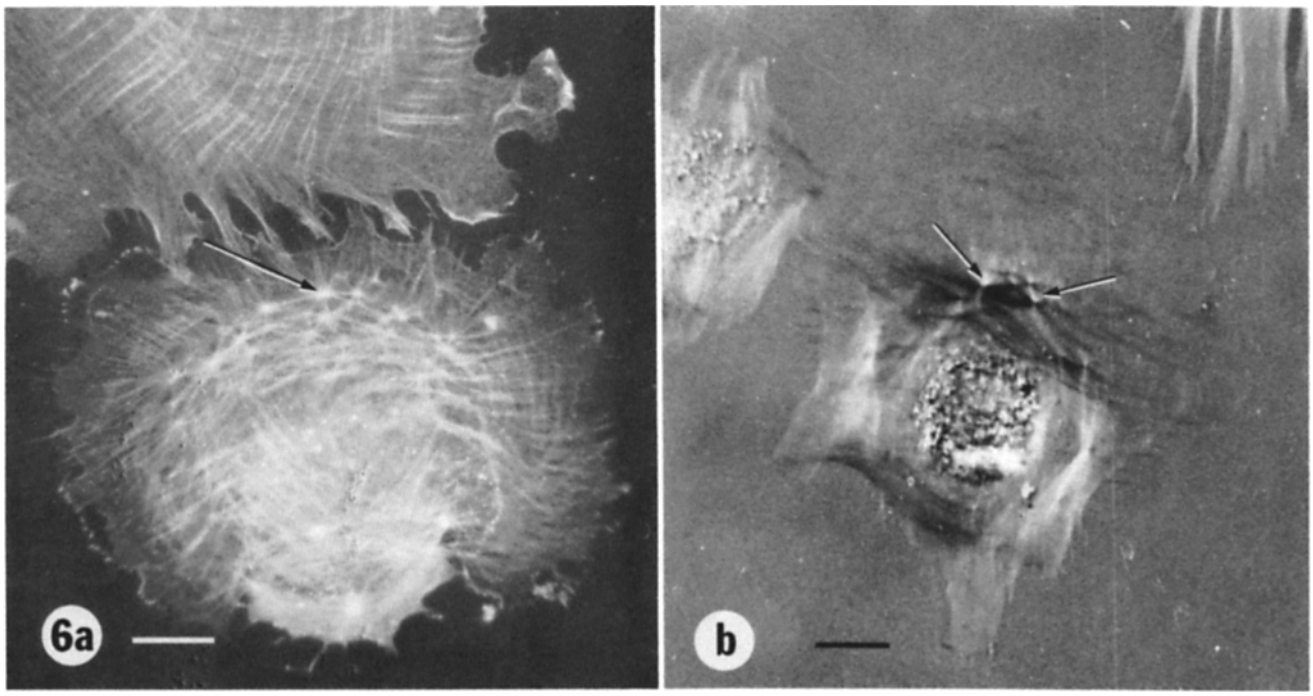


FIGURE 6 (*a*) Geometric patterns of actin-containing fluorescence structures observed in a cell stained with monoclonal antibody. The cytostructure of an adjacent cell (above) plated at the same time is in rotation. Filaments appear organized into nets or large polygons with dense fluorescence at the vertices (arrow) similar to those reported by Lazarides (24). (*b*) Image of a living cell in transition showing the polygonal net. Bright spots of birefringence (arrows) appear in the net at the vertices of polygons. (*a*) Bar, 10 μm; × 1,150. (*b*) Bar, 7.7 μm; × 1,300.

In some cells stained with actin antibody we observe fibers arranged in geometric patterns (Fig. 6*a*), similar to those reported in fixed cells stained with actin and other antibodies (17) and in living cells stained with 7-nitrobenz-2-oxa-1,3-diazole-phallacidin (18). Time-lapse recordings using polarization optics have shown that the formation of these patterns occurs late in cell spreading. As fibers begin to swirl, small bright spots of birefringence appear in the cytoplasm (Fig. 6*b*). These spots seem to be associated with linear birefringent structures. The nature of the association becomes clearer with time as the bright spots are seen as the vertices of triangular structures in a birefringent network. A number of such spots in the network can be seen in the photograph of a living cell in Fig. 6*b*. These spots linked by birefringent linear structures are observed to move in unison in time-lapse films as the cytostructure reorganizes.

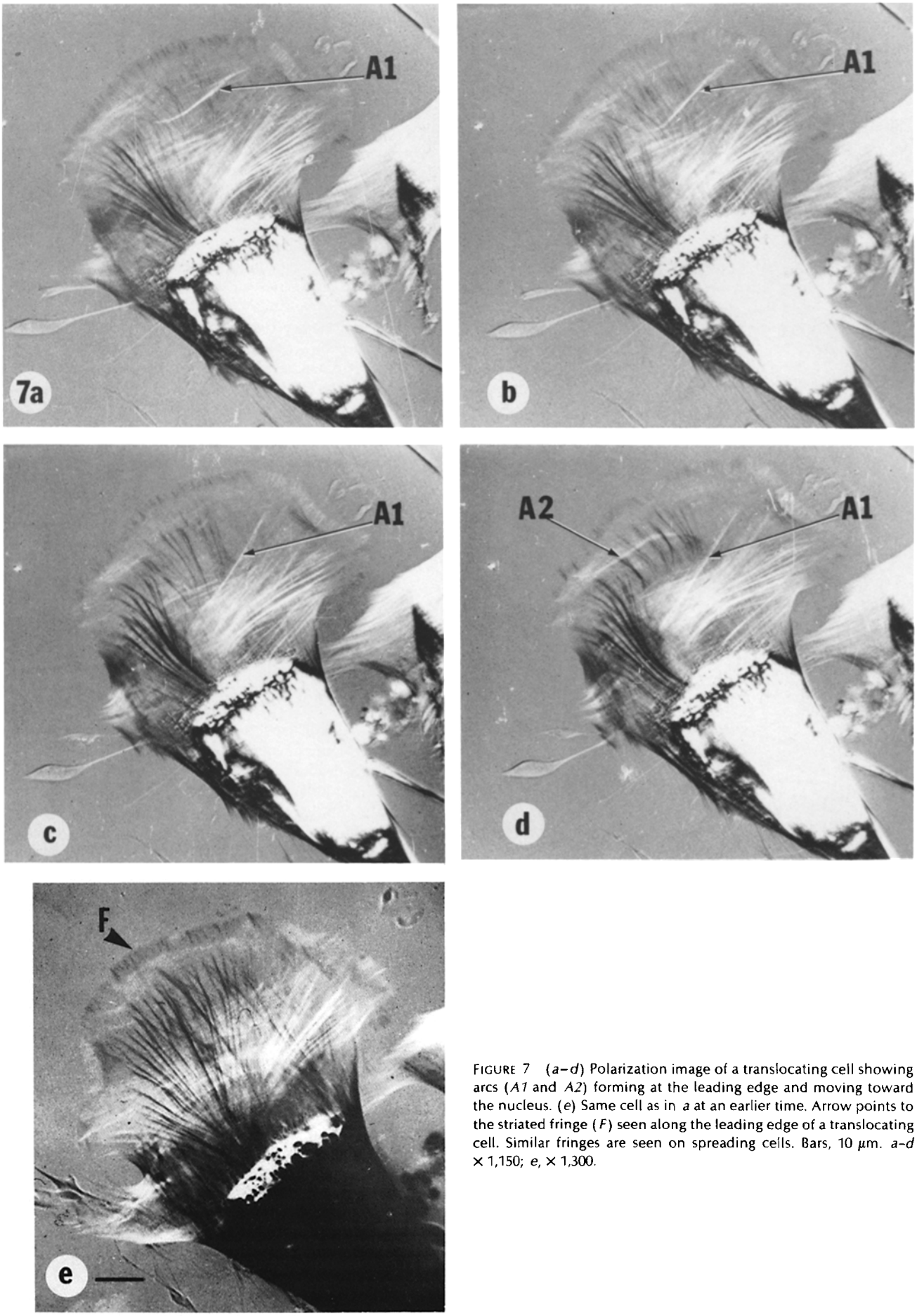
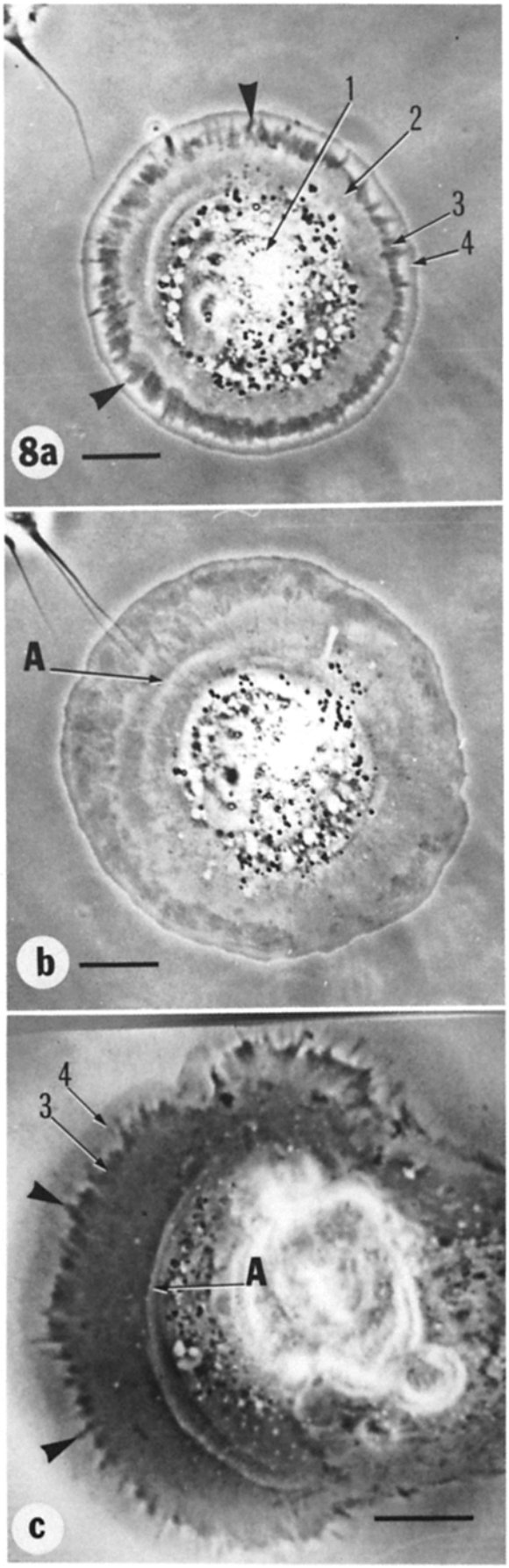


FIGURE 7 (*a–d*) Polarization image of a translocating cell showing arcs (*A1* and *A2*) forming at the leading edge and moving toward the nucleus. (*e*) Same cell as in *a* at an earlier time. Arrow points to the striated fringe (*F*) seen along the leading edge of a translocating cell. Similar fringes are seen on spreading cells. Bars, 10 μm. *a–d* × 1,150; *e*, × 1,300.



The cell in Fig. 6*a* was fixed at a stage similar to that in 6*b* and stained with actin antibody. Fibers can be seen arranged in a polyhedral net in which the vertices of the polygons appear strongly fluorescent.

Arc Formation and Movement in the Translocating Cell

Emanating from behind the fringe of the leading edge of the translocating cells is a continuous train of small arcs of birefringence that move steadily toward the nucleus (Fig. 7 *a–d*). These arcs seem to lie under sets of stress fibers which move as separate units. The positions of each set of three or four stress fibers change continuously. At the leading edge of the cell is an undulating or ruffling fringe 7.5 μm wide consisting of an array of parallel striae (Fig. 7*e*). On fixation with formaldehyde the fringe becomes scalloped and the striae disappear. When stained with antiactin antibody, the fringe stains uniformly with no striae detectable.

Phase-contrast Observations of Spreading and Translocating Cells

Some cytostructural changes are better revealed with phase-contrast than with polarization optics. In spreading and slowly translocating cells, four phase-contrast zones have been observed (Figs. 8*a* and *c*): a nuclear region, a phase-grey cytoplasmic region, a phase-black cytoplasmic region, and a circumferential phase-clear region extending from the phase-black area to the limiting membrane. In translocating cells the phase clear zone appears only in the region of the leading edge. At a time compression rate of 1–12 on a video recorder, a rhythmic percolation of the phase-black region into the phase-clear zone is observed. Continuous eruptions occurring along the edge of the phase dense zone appear to generate perturbations or waves that move toward the cell nucleus, but persist for only a few micrometers. The arcs described above begin to form slightly farther toward the cell nucleus.

As the edge of the phase dense area "boils," it also advances toward the limiting membrane. Once the phase clear zone becomes completely filled in (Fig. 8*b*), the cell extends its membrane, creating a new phase clear zone. In this manner a spreading cell appears to increase its circular area and a slowly translocating cell (Fig. 8*c*) moves it leading edge forward by discrete advances of the clear zone.

FIGURE 8 Phase-contrast images of a spreading and a translocating cell. (*a*) Four zones of contrast can be detected in a spreading cell: (*1*) a nuclear zone, (*2*) a phase-grey zone, (*3*) a phase-black zone, (*4*) a phase-white zone. The phase-black zone appears to percolate into the phase-white zone. Small eruptions (arrowheads) occur continuously while the cell is spreading. The phase-black zone advances into the white zone as it percolates. The white zone advances ahead of the black zone, but on occasion the black completely fills in the white zone, which may or may not reappear. At some point the cell no longer extends a white zone and both zones *3* and *4* disappear as seen in *b* (25 min later than *a*) in which a moving arc (*A*) is seen. (*c*) Image of a translocating cell which contains the four zones. Note that zones *3* and *4* appear only along the leading edge and not behind the nucleus. An arc (*A*) has formed in zone *2* and is moving toward the nucleus. (*a* and *b*) Bar, 5 μm; × 2,200. (*c*) Bar, 10 μm; × 1,450.

DISCUSSION

Arcs and circumferential bundles of filaments in spreading cells have been reported by investigators using either light microscopy (8, 9), electron microscopy (9–11), or immunofluorescent staining (19). In the present paper we have provided evidence that they are dynamic actin-containing structures. They are seen in virtually all spreading or translocating cells in our cultures of either IMR 90 or AG1519 human fibroblasts. They have been reported to occur in chick heart cells (8, 9) and in epithelial cells (10, 11). We think an arc-shaped filament bundle forms as a result of a local condensation of the actin network which is structurally tied to the cell membrane. Arcs form near the periphery of spreading and translocating fibroblasts just behind intense percolations in a phase-dark zone, and the condensations or contractions are transmitted toward the nucleus. The medium in which the arc is traveling is assumed to be a filamentous actin-containing network through which a compressional wave is transmitted. What is visible to the eye is the compressed region of the net, which is densely populated with filaments. The medium itself is not translocated from the cell periphery to the nucleus; only the zone of compression is seen to move as the wave progresses. Optical sectioning at the periphery shows the condensed rings lying close to the substrate side of the cell. They are also not far from the dorsal surface at the periphery because cell thickness there is small, ~2–3 μm in spreading cells and less in translocating ones. Because the condensations seen as arcs or circles of birefringence are actin rich, as we have shown by indirect immunofluorescence, we feel we have provided evidence they may be compressed regions of the subplasmalemmal actin network (20–30).

Micrographs of sections of spreading epithelial cells (10) cut perpendicular to the substrate show filament bundles in cross section lying on the nuclear side of an uplifted arc of ventral membrane. We interpret the filament bundles, as others have, as compressed volumes of the network. We do not rule out the occurrence of undulations associated with the dorsal surface as reported by Heath (9). Although the electron micrograph of a vertical section through a cell shows a small depression in the dorsal lamella, no underlying bundle of filaments is visible.

Many authors have suggested that a subplasmalemmal network (20–28, 31) might be involved in tissue cell motility. While the network is thought to consist mainly of 5–6-nm actin microfilaments sometimes organized into bundles, also present are intermediate filaments and microtubules to both of which the actin filaments attach, directly or through linkers (32). While the microtubules might provide the skeletal framework for the net, it is also apparent in spreading cells that radial stress fibers (Fig. 3) may serve a skeletal function. Since their length increases with increasing cell diameter, they may play a part in extending a spreading cell at the periphery. Where a radial stress fiber is intersected by an arc of contracted net, the stress fiber is bent into an angle (Figs. 1 *b* and 3), suggesting an attachment between the net and the radial stress fiber. The angle is visible only where there is lateral displacement of the stress fiber. Stress fibers that do not appear angular at the point of intersection with an arc may not be displaced laterally at the apex and are seen as unbent structures, even though they may be angled. Vertical sections cut through the zone of intersection should clarify the relationship. We can only guess that the deformation of the radial element is due to a force exerted by the contraction of the microfilament net. By optical sectioning we can see that the radial stress fiber forms an angle with the plane of the substrate to which the cell is attached. The vertex of the angle is at the cell periphery so that the fiber extends from the ventral toward the dorsal cell surface. This is essentially what others have reported (8, 23). That the condensed net and the region of stress fiber associated with it are in the same plane of focus, even deep in the cell, whereas the ends of the stress fibers are out of focus, may be additional evidence for a relationship between the two structures.

We suggest that movement of the arcs is a manifestation of a compressional wave traveling through a medium consisting of a microfilament net. Such a traveling compressional wave was posterlated by Ambrose (31). In compressed domains, elements of the net would be brought into proximity and conditions would exist for filament interaction. An active radial contraction might occur then in the region of compression, accentuating the condensation seen as an arc.

When a cell is fully spread, it begins shortly afterward to translocate, but before it does so it undergoes a major cytostructural transition. The arcs of birefringence that move from the cell periphery to the perinuclear zone disappear. Radial stress fibers elongate, are seen to rotate with the nucleus, and become spiraled as a consequence. They seem eventually to be thrown into a band of concentric rings at the cell periphery away from the nucleus. We suggest that the pressure developed by the growth of radial stress fibers attached to a perinuclear net rotates the nucleus.

It is at a stage after rotation has begun that we first observe birefringent geometric networks of actin-rich elements in living cells. These networks are not microfilament networks; rather, they are networks of bundles of fibers whose elements are approximately the diameter of radial stress fibers. It is possible that the latter subplasmalemmal network is recruited to provide the additional bundles of fibers whose ends disappear in the fine microfilament net (Fig. 6 *a*). The sequence of cytostructural changes beginning with ring or arc formation and leading to the reorganization of the radial stress fibers just before the onset of translocation is highly invariant and is seen in virtually all IMR 90 cells examined.

A growing radial stress fiber structure may be a necessary cytostructural condition for the spreading of lung fibroblasts, but it appears to break down after a cell undergoes the transition to a state of locomotion (Fig. 4 *h* and *i*). Although some locomoting cells are endowed with stress fibers, others, the fastest moving ones, appear to have few or none (2). In both spreading and translocating cells, however, we have observed moving arcs that may be associated functionally with both activities.

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