BEHAVIOUR AND STRUCTURE OF THE LEADING LAMELLA IN MOVING FIBROBLASTS

1. OCCURRENCE AND CENTRIPETAL MOVEMENT OF ARC-SHAPED MICROFILAMENT BUNDLES BENEATH THE DORSAL CELL SURFACE

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SUMMARY

Arc-shaped bundles of microfilaments are frequently found beneath the dorsal surface of the leading lamella of chick embryo fibroblasts. These structures, called arcs, form parallel to, and 2-10 μm from the leading edge of the lamella and they then move centripetally through the lamella and disappear in front of the cell nucleus. Arcs move centripetally at a mean speed of 1.33 (±0.08 s.e.) μm min⁻¹ relative to the substratum.

Arcs are specifically associated with cells that are actively protrusive. They are common in fan-shaped fibroblasts and in those cells that are newly spreading on a substratum. Arcs are absent from fibroblasts that have only small lamellae or that are polygonal. The correlation between arc formation and protrusive activity is also clearly shown by the locomotory behaviour of fan-shaped cells. When protrusion of the lamella is increased by tail detachment, fibroblasts often develop numerous arcs, which chase each other backwards through the lamella. Conversely, arc formation ceases during contact inhibition of locomotion.

In cells with large convex-edged lamellae there is a dorsal submembraneous sheath of microfilaments. All of the filaments comprising the sheath are oriented parallel to the margin of the lamella. Arcs are regions of the sheath where the microfilaments are more densely packed and hence visible by phase-contrast microscopy. There is no obvious relationship between the dorsally situated arcs and microfilament sheath, and the stress fibres that are associated with the ventral cell surface.

The similarities between the movement and behaviour of arcs and the centripetal transport of particles on the surface of the lamella suggest a role for the microfilament sheath in the movement of particles.

INTRODUCTION

The study of the locomotory behaviour of cultured cells has led to the identification of some of the molecular events involved in mechanisms of movement. The stress fibres, or microfilament bundles (Buckley & Porter, 1967), contain mechanochemical proteins and it is widely held that the contraction of these bundles (Isenberg et al. 1976) may contribute to the forces required to move fibroblastic and epithelial cells forward to their anterior adhesions with the substratum (Heath & Dunn, 1978; Abercrombie, 1980; Heath, 1982). However, the mechanisms that control the protrusive activities of a moving cell, in their many and varied forms, are less well understood. The extension of leading lamellae, ruffles, blebs, filopodia and microspikes from the edges of crawling cells are examples of the widely differing protrusive behaviour associated with movement. And yet it may be that these protrusions have
a common molecular basis involving changes in the polymerization state of actin (Wehland, Osborn & Weber, 1979; Small & Celis, 1978; Hoglund et al. 1980).

A behavioural feature that cellular protrusions have in common is centripetal movement. Ruffles, blebs and microspikes often move backwards for short distances over the dorsal cell surface. But the most striking example of centripetal movement in fibroblasts is the behaviour of arcs, which are a novel class of microfilament-based structures found in the leading lamellae of chick embryo fibroblasts (A. K. Harris, unpublished; Heath & Dunn, 1978; Dunn, 1980; Heath, 1981). Arcs are curved bands of microfilaments, which develop close to the leading edge of actively protrusive cells and then move rearwards through lamella and disappear near the nucleus. This paper analyses the correlation between arc formation and various aspects of the locomotory behaviour of chick embryo fibroblasts, and includes some details of the fine structure of arcs and their relation to the dorsal cell surface.

MATERIALS AND METHODS

Cell culture

Primary outgrowths of fibroblastic cells were obtained from fragments of 6- to 8-day-old chick embryo heart cultured on glass coverslips. The cultures were maintained in Medium 199, supplemented with 10% foetal calf serum, 100 units ml⁻¹ each of penicillin and streptomycin, and 2 mM-glutamine, at 37 °C in an atmosphere of 5% CO₂ in air, for 24–96 h.

Secondary cultures of fibroblasts were prepared as above except that the heart fragments were cultured in Falcon flasks. After 48 h, the outgrowths were rinsed in Ca- and Mg-free Hanks' saline, incubated in 0.05% trypsin in 0.02% EDTA for 5 min, and the fibroblasts were then suspended in fresh culture medium and seeded on to coverslips.

Using the same culture conditions, fibroblastic and epithelial cells were also grown from chick embryo cornea, sclera, intestine, dermis, muscle and kidney, and from neonatal mouse epidermis and muscle, and neonatal rat muscle.

Cinemicrography

The cultures were incorporated into stainless steel filming chambers containing fresh medium. The cell cultures were examined on a Zeiss Photomicroscope equipped with positive phase-contrast optics. The culture chambers were maintained at 37 °C with an air curtain incubator. Time-lapse recordings of cell behaviour were taken on Kodak 16 mm Plus-X Reversal film at 0.1–2 frames per second. Additional recordings were taken on video tape. An LW analytical projector was used for frame-by-frame analysis of cine film. Cell outlines, and the positions of arcs, were traced from the projected film. All area measurements were made with a planimeter.

Micromanipulation

The coverslip cultures were incorporated into the upper side of De Fonbrune glass chambers, one side of which was sealed with paraffin oil to allow the introduction of needles into the chamber. Glass microneedles with tip diameter 0.5–2.0 μm were drawn by hand in a microburner and mounted in a De Fonbrune micromanipulator. The microneedles were positioned next to the tip of the tail of a fibroblast; the tip was then gently prodded until it broke away from the substratum and was retracted towards the cell body.

Electron microscopy

For transmission electron microscopy (TEM) the cells were cultured on carbon-coated 22 mm diameter glass coverslips. The cells were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 3 mM-calcium chloride, at 37 °C for 1 h. After a brief rinse in buffer, the cells
were post-fixed in 1% osmium tetroxide in buffer for 1 h at room temperature, stained for 30 min in 0.5% uranyl acetate in water, dehydrated in ethanol and embedded in Araldite. After polymerization of the plastic, the coverslips were removed by inserting a razor-blade between the plastic and the carbon-coated coverslips; the fracture plane runs between the carbon and glass leaving the cells undamaged. The blocks were trimmed and horizontal sections were cut on an LKB Ultratome. Vertical sections of the cells were cut after a second layer of Araldite was polymerized on the exposed block face. Serial silver sections were picked up on slot grids and stained in uranyl acetate and lead citrate, and examined in a JEOL 200CX electron microscope at 80 kV.

For scanning electron microscopy (SEM) the cells were fixed as above and dehydrated in ethanol and acetone, critical-point dried from liquid CO2, sputter-coated with 20 nm gold, and examined in a JEOL scanning electron microscope.

RESULTS

Cell morphology and arc formation

An arc is a phase-dense curved fibre, which forms 2–10 μm from the margin of a well-spread fibroblast. Arcs can be clearly distinguished from the other linear dense structures of the leading lamella (such as stress fibres) because they possess two unique characteristics: they form subjacent to and parallel to the lamellar margin, and they move centripetally through the lamella eventually disappearing before they reach the nucleus (Fig. 1).

Arcs are found only in fibroblasts that have a large leading lamella. Examples of this morphological type of fibroblast are shown in Figs 1–4. Arcs occur most typically in fan-shaped fibroblasts of which Fig. 1 is an example. In these cells the lamella is roughly semicircular in outline, more than 20 μm wide and more than 15 μm from margin to nucleus. Other kinds of arc-forming cells include the large binucleated fibroblasts (Fig. 2), which are not infrequent in primary chick heart fibroblast (CHF) cultures, and cells that are respreading after dissociation and often have a 'fried egg' morphology (Figs 3, 4). But the salient features shared by arc-forming cells are: first, a large area of lamellar cytoplasm with convex margins; and second, a continual protrusive activity at the lamellar margin, although in the case of circular cells (Fig. 4) there is little or no net translocation of the cell until it develops a polarity.

In primary CHF cultures, fixed after 48 h, nearly one third (29.3%) of all the isolated and freely moving cells at the edges of the outgrowths have broad and roughly semicircular lamellae more than 20 μm wide (Table 1). And of these cells about one fifth (18.8%) contain one or more arcs within their lamellae. The rest of the cells at the outgrowth margins are narrow or else polygonal; in either case, these cells have smaller lamellae, less than 20 μm wide, and few if any areas of their margins are convex (Fig. 5). As Table 1 shows, no arcs were found in these morphological types of chick fibroblast.

After 72–96 h in culture, very few arcs are found among the fibroblast outgrowths from chick heart explants and nearly all the cells have adopted a spindly or polygonal morphology. But these cells can be induced to form arcs if they are treated with EGTA. Six 72-h-old CHF cultures were treated with 2 mM-EGTA for 10 min which caused the cells to round up but not detach. As the cells respread in normal culture medium they reverted to a circular or fan-shaped type. The cultures were fixed after
Table 1. Incidence of arcs in chick embryo heart fibroblasts

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Cell morphology</th>
<th>No. of cells counted</th>
<th>No. with arcs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Primary explant cultures 48 h-old</td>
<td>Spindly and polygonal with small lamellae (&lt;20\mu m wide)</td>
<td>899</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Fan-shaped with large lamellae (&gt;20\mu m wide)</td>
<td>373</td>
<td>70</td>
<td>18.8</td>
</tr>
<tr>
<td>B. Primary explant cultures 76-h-old, respreading after exposure to EGTA</td>
<td>Fan-shaped and circular</td>
<td>187</td>
<td>58</td>
<td>31.0</td>
</tr>
<tr>
<td>C. Secondary fibroblasts 90 min after seeding</td>
<td>Circular</td>
<td>138</td>
<td>47</td>
<td>34.1</td>
</tr>
</tbody>
</table>

Fig. 5. Phase-contrast micrographs showing the morphological types of fibroblasts in which arcs have not been found. A. An elongated fibroblast with a small lamella less than 20\mu m wide. B. A polygonal fibroblast with no convex margins. Bar, 20\mu m.

Figs 1–4. Phase-contrast micrographs of chick heart fibroblasts. Each cell contains an arc within its leading lamella. Bars, 20\mu m.

Fig. 1. A. A fan-shaped primary fibroblast in a 48-h-old explant culture. The arc is a phase-dense fibre, which lies parallel to the margin of the lamella and at right angles to the direction of cell movement. B. The same cell 3 min later. The arc has moved rearwards towards the nucleus, meanwhile a second arc has formed in the right-hand region of the lamella (arc").

Fig. 2. A large binucleated primary fibroblast. The arc can be clearly distinguished from the stress fibres (sf). Arcs always lie parallel to the lamellar margin; stress fibres are oriented in the direction of cell movement.

Fig. 3. A newly spreading secondary fibroblast, 90 min after seeding. An arc has just formed and appears as a curved phase-bright structure (arrow) subjacent to the lamellar margin.

Fig. 4. A circular secondary fibroblast, 90 min after seeding. Such cell types are continually protruding and retracting their lamellar margins, but there is little net translocation. Note the arc within the lamella on the left.
10 min of respreading and the cells were examined for arcs; of 187 cells examined, 58 (31·0 %) contained arcs. This increased incidence of arcs in respreading cells is also seen if the fibroblasts are totally detached and then seeded on to a fresh coverslip. As the fibroblasts spread out on their substratum, and before they achieve a polarized form, they usually go through a stage in which they have a circular form with a large area of lamellar cytoplasm (Figs 3, 4). A large proportion (34·1 %) of these cells also contain arcs (Table 1).

**Arc formation and movement**

The main statistics of arcs are listed in Table 2. Arcs are first seen a mean distance of 7·75 μm from the leading edge of the lamellae of chick fibroblasts. Arcs always form rapidly, usually within 20 s. In positive phase-contrast optics, an arc is first noticed as a curved, linear phase-bright structure in an otherwise uniformly grey lamella. They always develop on the dorsal, i.e. medium-facing, surface of the lamella. Within a minute the bright structure reaches maximum size and develops into a curved phase-dense fibre, usually 5–20 μm long and 0·5–1·5 μm wide, with a phase-bright region of similar proportions on its inner side (Figs 1–4, 7). The bright region of an arc is not in fact part of its structure, as is explained below, but is the most obvious sign of a newly forming arc when the cells are viewed by phase-contrast microscopy.

Very large arcs, up to 60 μm in length, are sometimes found in the lamellae of binucleated fibroblasts, which typically have three to four times the area of lamellar cytoplasm of uninucleated cells (Fig. 2). The differences in shape and position between arcs and stress fibres are most clearly seen in these large binucleated cells (Fig. 2).

An arc is not always a perfect segment of a circle; its radius of curvature may vary along its length but generally conforms to that of the margin of the lamella at the time when the arc develops (e.g., see Figs 7, 12).

Immediately they appear, and often before they have developed to full size, arcs start to move centripetally through the lamella. It is this characteristic motility of arcs that clearly distinguishes them from other phase-dense structures of the lamella. Arcs move rearwards with respect to the cell and to the substratum. The centripetal movement is shown graphically in Fig. 6, and in the sequences of photographs in Figs 1 and 7. In the cell depicted in Fig. 6, the lamella and nucleus advance at speeds of 0·3 and 0·6 μm min⁻¹, respectively, whereas the arc travels backwards at nearly 1·0 μm min⁻¹. Measurements from time-lapse films of the distances travelled between

<table>
<thead>
<tr>
<th><strong>Table 2. Characteristics of arcs</strong></th>
<th>Mean (±s.e.)</th>
<th>No. of arcs</th>
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<tbody>
<tr>
<td>Distance from lamellar margin when first seen</td>
<td>7·75 (±0·62) μm</td>
<td>31</td>
</tr>
<tr>
<td>Distance moved through lamella</td>
<td>7·12 (±0·63) μm</td>
<td>43</td>
</tr>
<tr>
<td>Speed through lamella</td>
<td>1·33 (±0·08) μm min⁻¹</td>
<td>43</td>
</tr>
<tr>
<td>Speed during first minute after formation</td>
<td>1·88 (±0·10) μm min⁻¹</td>
<td>34</td>
</tr>
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*Distances and speeds are relative to the substratum.*
formation and disappearance of 43 arcs in 20 fibroblasts gave a mean speed of arcs of \(1.33 \mu m \text{ min}^{-1}\) with respect to the substratum (Table 2). As Fig. 6 shows, arcs gradually decelerate as they move near the nucleus; their mean initial speed, taken as the distance travelled in the first minute after formation, is \(1.88 \mu m \text{ min}^{-1}\). In a single instance, an arc was recorded with an initial speed of \(3.85 \mu m \text{ min}^{-1}\).

Arcs move rearwards a mean distance of \(7.12 \mu m\) through the lamella; their travel terminates over the perinuclear zone of organelles. In large binucleated fibroblasts arcs may move up to \(40 \mu m\) between formation and disappearance. As arcs move rearwards the phase-bright region gradually fades leaving a phase-dense fibre to continue on to the perinuclear zone (Fig. 7). Careful focusing with a high-power objective shows that a newly formed arc demarcates an abrupt change in lamellar thickness. This change is more clearly seen by SEM and TEM (Figs 13, 14). The sudden fall in lamellar thickness, coupled with the high refractive index of the arc itself, most probably generates a bright 'phase halo' on the inner side of the arc. The

![Graph](image)

**Fig. 6.** Centripetal movement of an arc through the lamella of a chick fibroblast. Data taken from a time-lapse movie of a cell similar to that shown in Fig. 7. At time 0 an arc (A) formed within the lamella (L). A line was drawn parallel to the direction of cell movement passing through the midpoint of the arc, and through the margin of the lamella and the anterior edge of the cell nucleus (N). The movement of these three structures along the reference line was plotted at 1 min intervals for the lifetime of the arc, a total of 10 min. During this period the margin of the lamella protrudes forward at \(0.3 \mu m \text{ min}^{-1}\), and the nucleus moves forward at \(0.6 \mu m \text{ min}^{-1}\). The arc, which formed 9 \(\mu m\) from the lamellar margin, moves rearward, with respect to the substratum, at a mean speed of \(0.9 \mu m \text{ min}^{-1}\). The arc disappears in the perinuclear zone about 7 \(\mu m\) in front of the nucleus.
Centripetal movements in fibroblasts

The halo effect of phase-contrast microscopy is especially prominent at boundaries between regions giving markedly different phase changes (Ross, 1967). When an arc reaches the rear of the lamella there is no change in lamella thickness and the phase-bright region is much reduced or absent (Fig. 7).

On reaching the perinuclear zone arcs rise away from the substratum, following the contours of the upper surface of the fibroblasts (see Fig. 13). As they move over the perinuclear organelles their image gradually fades and always disappears before the arcs have reached the nucleus (Fig. 7).

**Frequency of arc formation**

By following the movements of individual fan-shaped cells for periods of an hour or more it was found that the rate at which arcs form in fibroblasts with similar morphologies and with similar rates of movement can vary considerably. Many cells with large leading lamellae did not form any arcs during the periods of observation; by contrast, other fibroblasts formed arcs almost continuously. Fig. 7 shows a fan-shaped chick fibroblast in which there is a continuous procession of arcs through its lamella over a period of 9 min. In a cell such as this arcs may form and move independently in different parts of the lamella. The shortest recorded interval between two arcs in the same part of a lamella was 50 s and occurred in the cell shown in Fig. 8.

Although it is not clear what initiates the formation of an arc, the analysis of the films showed that there are at least two aspects of fibroblast locomotory behaviour that can have substantial effects on the incidence of arcs: tail detachment was found to promote arc formation, and cell–cell contact inhibited arc formation.

**Arcs and tail detachment**

During their normal locomotion on a plane substratum, chick fibroblasts often undergo spontaneous tail detachments. The trailing regions of the cell, or tail, will suddenly detach from the substratum and be withdrawn or retracted into the centre of the cell. Tail detachment can also be induced by micromanipulation. Detachment almost invariably leads to an increase in the rate of protrusion of the lamella, of as much as three times the normal rate for a period of about 10 min (Fig. 8). This behaviour is known as retraction-induced spreading (RIS) (Chen, 1979) and it has been the subject of detailed study in this laboratory and elsewhere (Chen, 1981a, b; Dunn, 1980; Heath & Dunn, unpublished data).

It was noticed that tail detachment (TD) in chick fibroblasts is followed not only by an increase in the protrusive activity of the lamella but also by an increase in the

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**Fig. 7.** Sequence of phase-contrast micrographs of primary chick heart fibroblast taken at 1 min intervals and showing the centripetal movement of a succession of arcs in the lamella. At time 0 an arc is just forming (arrow). By 2 min it has developed into a phase-bright line nearly 20 \( \mu \)m long and has moved rearward with respect to the substratum. At 3 min the arc can be seen as a phase-dense fibre with a bright phase halo on its inner margin. At 5 min the bright region has gone leaving just a phase-dense fibre. At 6 min the arc has reached the perinuclear zone and is fading. This cell produces 5 arcs, one after the other, during the 9 min period. Bar, 20 \( \mu \)m.
Fig. 8. Correlation of the locomotory behaviour of primary chick fibroblast with the incidence of arcs in its lamella. The cell outline was traced from a film, and the changes in cell shape, area and rate for protrusion were measured over a period of 96 min. As the cell moved forwards it underwent four tail detachments, which led to the tail being retracted into the cell body (marked by asterisks). The times at which arcs first appeared in the lamella are indicated by vertical bars. The cell is protruding at a basal rate of around 75 \( \mu \text{m}^2 \) per 2-5 min, but following each tail retraction the protrusive activity increases as much as 2-5 times normal for about 10 min. Note that there are some other smaller fluctuations in protrusive activity. Comparison of the rate of arc formation with the locomotory behaviour of this cell does not, however, reveal any obvious correlations. The intervals between arcs are distributed exponentially, which suggests that the arcs occur randomly.

formation of arcs. A number of films, taken during a study of TD and RIS in chick fibroblasts (Heath & Dunn, unpublished data), were used to analyse the association between TD and arc formation. A total of 21 cells were selected from the films as being suitable for this analysis. Each had been filmed for 10 min or more around the time when they were induced into TD by micromanipulation. The results are shown in Fig. 9. The horizontal bars show the periods the cells were filmed before and after TD; the small vertical bars indicate the formation of arcs.

Thirteen (62\%) of the cells formed one or more arcs before TD, but every cell formed one arc, and usually more, after TD. The mean frequency of arc formation before TD in all cells was 0.122 arc min\(^{-1}\). During the 10 min period after TD, i.e. in the phase of increased protrusive activity (RIS), the frequency rose to 0.177 arc min\(^{-1}\), an increase of 45\%; from 10 min onward, when protrusion had fallen back to normal levels, the frequency of arc formation dropped to 0.14 arc min\(^{-1}\). The effect of TD on arc formation is also shown by very significant difference, in 13 cells, of the
Centripetal movements in fibroblasts

Fig. 9. Increase in arc frequency caused by retraction-induced spreading. Each horizontal bar marks the durations before and after tail detachment (TD) for which a cell was filmed. The small vertical bars mark the times at which each cell formed an arc. Following TD, all of these cells showed an increased rate of protrusive activity for 10 min, which coincided with an increase in the frequency with which the cells formed arcs.

lengths of the period between the last arc and TD, and TD and the next arc. In a paired \( t \)-test, the lengths of the periods between TD and the next arc were significantly shorter \( (t = 2.82, \text{ d.f.} = 12, P < 0.02) \). This significance cannot be due to the bias introduced by the selection of the 13 cells for this test, which would, because of the generally shorter period of filming before TD, tend to produce the opposite result.

In the cells analysed above there was just a single tail detachment during the period of filming. On a few occasions fibroblasts that underwent a series of spontaneous tail detachments were observed, and the film taken of one of these cells was analysed frame-by-frame to see if the increase in arc formation was repeated after each successive TD. The incidence of arc formation was compared with three other parameters: cell shape, area and protrusion rate; the results are shown in Fig. 8. The cell was filmed for 96 min; during this period it underwent four tail detachments and formed 47 arcs. As expected, there is a marked increase in the protrusion rate for 5–10 min after each TD; the rate increases by as much as 2.5 times the basal rate of 75 \( \mu \text{m}^2 \) per 2.5 min interval. It was also found that the normal protrusion rate fluctuated in between detachments. But when the occurrence of arcs was compared with the four parameters of locomotory behaviour no clear pattern could be found. Indeed, the most that can be said is that arcs appear to be random events, since a statistical
analysis of these data revealed that the intervals between arcs closely follow the cumulative exponential distribution (Colquhoun, 1971, chap. 5) (graph not shown).

**Contact inhibition of locomotion**

The results presented so far have all pointed to the association of arc formation with the protrusive activity of the lamella. A familiar situation in which lamellar protrusion is inhibited occurs when two fibroblasts collide and undergo contact inhibition of locomotion. On six occasions, collisions occurred between cells that were forming arcs continually prior to the collision. The resulting inhibition demonstrated unequivocally that new arcs do not form in areas of the lamella that are not protruding.

Fig. 10 shows a representative case. The outlines of two colliding cells are shown as they undergo the classical reaction of contact inhibition of locomotion. Before contact, arcs form throughout the lamella of the upper cell. While the cells are in contact arcs form only in the free, i.e. non-inhibited, areas of the lamella. Later, a new

![Fig. 10. Sequence of tracings from a film showing contact inhibition of locomotion and its effect on the formation of arcs in a fibroblast. The cross marks a stationary reference point on the substratum. Times are given on the right in minutes and seconds. The positions of arcs in the lamella of the upper cell were traced in each frame of the sequence. Note that arcs occur continually until contact is established. Arc formation is inhibited in those parts of the lamella in which further protrusion is stopped by contact with the lower cell, but is re-established when a new lamella is formed and protruded in a direction away from the region of cell-cell contact. Bar, 25 μm.](image-url)
Centripetal movements in fibroblasts

A well-characterized phenomenon of the behaviour of cultured fibroblasts is particle transport on the dorsal cell surface (Abercrombie, Heaysman & Pegrum, 1970b; Harris & Dunn, 1972). When moving cells encounter small particles, of varying nature, the particles frequently adhere to the edge of the lamella and are then transported centripetally on the dorsal cell surface at mean speeds of around $2.03 \mu m \text{ min}^{-1}$ (Abercrombie et al. 1970b) to $3.33 \mu m \text{ min}^{-1}$ (Harris & Dunn, 1972).

During this study it was observed that in seven fibroblasts (quite a large number in view of the rarity of the events occurring simultaneously) a particle that each cell picked up from the substratum had moved backwards a distance of about 3-5 $\mu m$ from the margin of the lamella when an arc formed directly beneath it, and then the particle and arc moved together over the lamella until the arc disappeared in the perinuclear region. Interestingly, in none of the seven cases did the particle overtake the arc, suggesting that some kind of transmembrane association constrained the particle and the arc to move backwards together.

Two cases of particles and arcs moving together occurred in the cell depicted in Fig. 11. This cell was forming arcs almost continuously and, as it encountered and picked up two particles of debris from the substratum, the particles were carried centripetally lying on top of two of the arcs.

Cell-to-substratum contacts

As mentioned earlier, arcs form on the dorsal surface of fibroblasts and so it was not expected that arcs would be involved in the contacts made between the cells and their culture substratum. This was confirmed by interference reflection microscopy (IRM). Fig. 12 shows a fan-shaped fibroblast viewed by phase-contrast and IRM. The cell has the pattern of cell-to-substratum contacts typical of a primary CHF (Izzard & Lochner, 1976), but there is no structure within the region of close and focal contacts that corresponds directly to the arc. However, the position of the arc can be gauged from the IRM image because the lamella anterior to the arc has a lower reflectivity than that behind it. This sudden change IR image corresponding with the position of an arc has been seen in nearly every other cell examined to date and it may be related to the increased thickness of the lamella in front of an arc.

Fine structure of arcs

In the scanning electron microscope the most striking feature of the lamella of arc-containing cells is the increased thickness of the lamella anterior to the arc. This thickening, which occurs in both fan-shaped and respreading cells, is seen in Figs 13 and 14. The lamellar thickening was also clearly seen by transmission electron microscopy of vertical sections of fibroblasts (see fig. 4 of Heath, 1981). But vertical sections did not provide any useful information on the structure of arcs; a clearer picture was obtained from sections cut parallel to the substratum.
Fig. 11. Association between arc movements and the transport of particles on the dorsal cell surface. Sequence of tracings taken from the film of the fibroblast shown in Fig. 8. Times are given in minutes and seconds from the first tracing; the cross is a stationary reference point. At time 0:00, two arcs, A1 and A2, are moving centripetally through the lamella; a particle P1 is also moving back on top of and at the same speed as A2. A second particle P2 lies on the substratum in front of the cell. By 4:18, A2 and P1 have moved rearwards together, preceded by A1, which has now disappeared in front of the nucleus. At 10:12, the lamella encounters P2 and A3 is moving through the lamella. By 13:54, P2 has been picked up and transported rearwards lying on top of a newly formed arc A4. At 16:00, both particles have come to rest in front of the nucleus, while A5 has developed and is moving back through the lamella. Bar, 20 μm.

Fig. 12. Phase contrast (a) and interference reflection (b) micrographs of a fan-shaped fibroblast with an arc in its lamella. The IR image in b was taken 2 min after a, when the arc has moved further rearwards in the lamella. Note the lower reflectivity of the lamella anterior to the arc. Bar, 20 μm.
A complete series of horizontal sections were cut through each of eight fibroblasts. From these, three-dimensional reconstructions of the whole lamella were made for each cell. What follows is only a short description of the major structural features of arcs; a more detailed ultrastructural and immunocytochemical study of arcs is in progress and the results will be published separately.

Figs 15 and 16 are consecutive sections through the lamella of a spreading fibroblast. They are, respectively, the fifth and sixth sections from the substratum and lie approximately 325 nm and 400 nm from the ventral surface of the lamella. In every cell examined to date the arc is found lying just beneath the dorsal surface of the lamella. Arcs are composed of a band of actin microfilaments of 5 nm diameter. But arcs are not discrete structures; they lie within, and are part of, a continuous sheath of circumferentially oriented microfilaments that lies on the dorsal side of the lamella (Fig. 16). The orientation of the sheath microfilaments follows that of the lamellar margin in a fan-shaped cell. Thus an arc can be defined as a narrow region of the microfilament sheath where the filaments are packed more densely than usual.

The microfilament sheath contains randomly spaced densities, which are about 250 nm long by 100 nm wide. In some cells these densities are spaced more regularly with a periodicity of between 0.5 and 1.0 \( \mu m \). Microtubules and 10 nm intermediate filaments lie beneath the sheath, but they do not appear to be a major component of the sheath and they are frequently oriented at right angles to the microfilaments. Deeper within the lamella lie the main microfilament bundles of the lamella, the stress
Figs 14 and 15. For legend see p. 348.
Fig. 16. For legend see p. 348.
fibres, that pass obliquely backwards from the ventral surface to the perinuclear zone. However, there is no obvious connection between the stress fibres and the arcs or the microfilament sheath (Fig. 15).

A number of fan-shaped fibroblasts that lacked arcs, as judged by phase-contrast microscopy of the living cells before fixation, were also sectioned for TEM. The dorsal cortical microfilament sheath was also present in these cells. Furthermore, in some cells the sheath contained arcs, usually less than 100 nm wide, but of similar lengths to those arcs described above. Obviously, these smaller arcs were not visible by light microscopy. This finding raises the possibility that arcs are more numerous in chick fibroblasts than is indicated by phase-contrast observations alone.

Arcs in other cell types

This paper describes the behaviour of arcs in chick embryo heart fibroblasts, but, clearly, if arc formation is of importance in the mechanisms of cell locomotion it is necessary to explore whether the phenomenon is shared by other types of cell. Accordingly, a small range of fibroblastic and epithelial cells from other species were filmed to see if they produced arcs. The results are given in Table 3.

As expected, fibroblasts cultured from five tissues, other than heart, of the chick embryo also demonstrated arcs. But nothing identical to the arcs of chick fibroblasts has been discovered in the range of mouse, rat, hamster and human cells examined so far (Table 3). However, some of these cell types did form linear, phase-bright structures in their leading lamellae; but in no case did these structures demonstrate rearward movement nor did they develop into a phase-dense fibre.

A search of recent literature revealed a number of reports on the intracellular distribution of mechanochemical proteins, which included immunofluorescence or electron micrographs of cells containing curved arc-like structures in their lamellar cytoplasm. Brief details of these reports are included in Table 3 but it must be emphasized that at present there is no evidence other than structure and position to indicate that they are similar to the arcs of chick fibroblasts. The only published account of centripetal movements of microfilaments beneath the dorsal cell surface, based on time-lapse films, is that of Ireland & Voon, who showed that polygonal

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Fig. 14. Scanning electron micrograph of a circular secondary fibroblast showing the increased thickness of the anterior region of the lamella in the lower half of the cell. Bar, 10 μm.

Figs 15, 16. Transmission electron micrographs of consecutive horizontal sections through the dorsal regions of a fibroblast similar in shape to that shown in Fig. 14. They are, respectively, approx. 325 nm and 400 nm from the substratum. In Fig. 15 the section passes through the nucleus (n), cuts the stress fibres (sf) obliquely, and then passes through the arc and then out through the lamellar margin (lm). The arc comprises a band of actin microfilaments oriented parallel to the lamellar margin and at right angles to the stress fibres. Note the high density of polysomes (p) in the anterior region of the lamella. In the next section (Fig. 16) the plane of section passes through the nucleus and perinuclear organelle zone, through the dorsal cortical layer of microfilaments (mf) and out of the dorsal cell surface (arrow) and then through the arc and the thickened anterior region of the lamella. Bars, 1 μm.
Table 3. Occurrence of arcs in different cell types

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>Cell type</th>
<th>Arcs†</th>
<th>Arc-like structures‡</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick</td>
<td>Heart</td>
<td>Fibroblasts</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dermis</td>
<td>Fibroblasts</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intestine</td>
<td>Fibroblasts</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>Fibroblasts</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>Fibroblasts</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sclera</td>
<td>Fibroblasts</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cornea</td>
<td>Epithelial</td>
<td>-</td>
<td>+</td>
<td>Ireland &amp; Voon (1981), fig. 1</td>
</tr>
<tr>
<td></td>
<td>Hypoblast</td>
<td>Epithelial</td>
<td>-</td>
<td>+</td>
<td>Ireland &amp; Voon (1981), fig. 1</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>Epithelial</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spinal ganglia</td>
<td>Neurones</td>
<td>-</td>
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<tr>
<td>Mouse</td>
<td>Dermis</td>
<td>Fibroblasts</td>
<td>-</td>
<td>+</td>
<td>Wehland et al. (1979), fig. 5a</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>Fibroblasts</td>
<td>-</td>
<td>+</td>
<td>Vasiliev &amp; Gelfand (1976), fig. 2d</td>
</tr>
<tr>
<td></td>
<td>Epidermis</td>
<td>Keratinocytes</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Dermis</td>
<td>Fibrosarcoma FS9</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Embryo</td>
<td>3T3</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Embryo</td>
<td>Fibroblasts</td>
<td>-</td>
<td>+</td>
<td>Lazarides (1976b), fig. 5</td>
</tr>
<tr>
<td>Rat</td>
<td>Muscle</td>
<td>Fibroblasts</td>
<td>-</td>
<td></td>
<td>Wehland et al. (1979), fig. 5a</td>
</tr>
<tr>
<td></td>
<td>Mammary adeno-carcinoma</td>
<td>Epithelial</td>
<td>-</td>
<td>+</td>
<td>Lazarides (1976b), fig. 5</td>
</tr>
<tr>
<td></td>
<td>Embryo</td>
<td>Fibroblasts</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>Kidney</td>
<td>BHK-21 fibroblasts</td>
<td>-</td>
<td>+</td>
<td>Albertini &amp; Anderson (1977), fig. 8</td>
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<td></td>
<td></td>
<td>Nil-8</td>
<td>-</td>
<td>+</td>
<td></td>
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<td>Rabbit</td>
<td>Ovary</td>
<td>Granulosa cells</td>
<td>-</td>
<td>+</td>
<td>Haemmerli et al (1982), figs 5, 8</td>
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<td>Human</td>
<td>Tongue</td>
<td>Carcinoma cells</td>
<td>-</td>
<td>+</td>
<td>Hoglund et al. (1980), fig. 2b, c</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>Glial cells</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>WI38 fibroblasts</td>
<td>-</td>
<td>+</td>
<td>Lazarides (1976a), fig. 23</td>
</tr>
<tr>
<td></td>
<td>Cervix</td>
<td>HeLa</td>
<td>-</td>
<td>+</td>
<td>Hermann &amp; Pollard (1981), fig. 4a</td>
</tr>
</tbody>
</table>

* Based on author's own observations unless indicated otherwise by references.
†† Indicates the presence (+) or absence (−) of curved fibres, which move centripetally through the lamella.
‡‡ Indicates the presence of phase-bright structures, or arc-like bands of actin microfilaments in the lamella.
networks of microfilaments moved back beneath the surface of chick embryo hypoblast cells at speeds of around 0.2 μm min⁻¹ (Ireland & Voon, 1981, fig. 14).

DISCUSSION

Arcs and cell motility

This paper describes a novel type of microfilamentous structure, found in the lamellae of chick embryo fibroblasts, that is a product of an actively protrusive cell and that shares, along with lamellar processes such as ruffles and blebs, the property of centripetal flow. A clear correlation between arc formation and the protrusive activity of fibroblasts was demonstrated in four ways: by the presence of arcs in fan-shaped and respreading cells, by the promotion of arcs after tail detachment, by the inhibition of arc formation during contact inhibition of locomotion and, finally, by the absence of arcs in non-protruding polygonal cells.

At present, it is not apparent what contribution, if any, arcs make to the mechanisms of cell locomotion. Their position beneath the dorsal cell surface makes it unlikely that arcs are directly involved in exerting traction on the substratum. Furthermore, the formation of an arc occurs submarginally and, as Fig. 8 shows, seems unlikely to drive lamellar extension. Indeed, the fact that many fan-shaped cells move quite well without arcs, as judged by light microscopy, indicates that arcs are probably not necessary for movement. Perhaps arcs can best be regarded as a consequence of the process that controls the protrusion of the leading lamella of a fibroblast and, as such, the interest in studying arcs lies in what information their occurrence and behaviour can provide concerning the mechanisms of protrusion and, equally, the turnover of the microfilament system during locomotion. For example, arc formation appears to involve the lateral aggregation or bundling of sheath microfilaments; if so, then we have here a system for studying, and perhaps manipulating at a molecular level, the development of microfilament bundles in living cells.

Structure and position of arcs

Arcs appear to be an integral part of a dorsal submembraneous sheath of microfilaments that is a characteristic of fan-shaped fibroblasts. Further work is necessary to determine the structure and molecular composition of arcs, but preliminary studies indicate that arcs are similar to the stress fibres or microfilament bundles that are associated with the ventral cell surface. The cytoplasm of cultured cells is permeated by a meshwork of actin microfilaments (Webster, Henderson, Osborn & Weber, 1978), but most of the filaments are concentrated into a layer beneath the dorsal and ventral cell surfaces (Buckley & Porter, 1967; Wessells et al. 1971). Most attention has been paid to the organization of the ventral microfilaments and their involvement in cell–substratum adhesion and force generation during locomotion (Abercrombie, Heaysman & Pegrum, 1971; Goldman, Schloss & Starger, 1976; Rees, Lloyd & Thom, 1977; Heath & Dunn, 1978; Abercrombie, 1980), with the result that the dorsal microfilament layer has been largely ignored. However, as this and a number
Centripetal movements in fibroblasts

of other studies have shown, there can be a high degree of order of the microfilaments and associated proteins beneath the dorsal surface. Zigmond, Otto & Bryan (1979) described a dorsal submembraneous sheath of actin filaments and small bundles, oriented along the longitudinal axis of human fibroblasts, which stained with anti-myosin, anti-α-actinin and anti-tropomyosin antibodies giving a pattern of periodicities like that found in the stress fibres. In a stereo immunofluorescence microscopic study, Osborn & Weber (1979) detected a similar organization of microfilament bundles beneath the dorsal surface of rat mammary cells. What contractile or structural functions these dorsal arrays of contractile proteins have is not clear, but the behaviour of arcs suggests that their assembly and organization can be influenced by the locomotory activities of the cell.

Centripetal flow

The most striking feature of the behaviour of arcs is their rearward movement through the lamella. Arcs are yet another example of the phenomenon of centripetal flow; the rearward movement of cytoplasmic and surface-associated structures from the margin of the lamella towards the nucleus. The centripetal movements of ruffles, blebs, particles and other structures are a consistent feature of the locomotory behaviour of a wide variety of cells (Ingram, 1969; Abercrombie et al. 1970a,b; Harris & Dunn, 1972; Harris, 1973; Bray & Bunge, 1973; Dipasquale, 1975a,b). A variety of mechanisms have been proposed that explain some or all of these movements in terms of a general continuous rearward flow of the cell surface (Abercrombie et al. 1970b, 1972; Harris, 1973, 1976; Bray, 1973), of membrane lipid (Bretscher, 1976), of submembraneous material (Dembo & Harris, 1981), or a generalized centripetal continuous contraction of the microfilament meshwork within the lamella (Dunn, 1980).

Most of the centripetal movements of cytoplasmic structures during cell locomotion start at or close to the margin of the lamella and do not usually involve more than the anterior third of the lamella. Abercrombie et al. (1970a) found that the average movement rearward of all ruffles on chick and mouse fibroblasts was only 1·3 μm at a mean speed of 1·9 μm min⁻¹; however, ruffles that formed submarginally, and then moved backwards, travelled at a mean speed of 2·8 μm min⁻¹ for a mean distance of 7 μm. Pinocytotic vesicles, which form when ruffles collapse back into the dorsal surface of the lamella, also move centripetally at a mean speed of 3·4 μm min⁻¹ for a mean distance of 5·8 μm (Abercrombie et al. 1970b). Much of the centripetal flow of ruffles, blebs and microspikes on chick gut and corneal epithelial cells occurs within the first 2–5 μm of the lamella at speeds around 3 μm min⁻¹ (Dipasquale, 1975a).

Arcs fall into this pattern of centripetal flow but, more importantly, they provide convincing evidence that the flow does not occur only in the anterior part of the lamella but continues right back to the nucleus and so involves the whole of the lamella.

Particle transport

An intriguing finding during this study was that the movement of arcs was correlated with the centripetal transport of particles on the dorsal surface of the lamella.
Moreover, the behaviour of arcs has more in common with particle transport than with other examples of centripetal movement discussed above.

On chick corneal and gut epithelial cells, latex particles travel at mean speeds of 1.75 and 1.5 μm min⁻¹, respectively (Dipasquale, 1975a). Harris & Dunn (1972) observed the transport of anion-exchange resin particles on chick fibroblasts and showed that they reach a maximum speed of 3.3 μm min⁻¹ within 1 μm of the margin, but then decelerate at around 0.25 μm min⁻² as they move back. Abercrombie et al. (1970b) obtained almost exactly the same value for the mean speed of carbon particles on non-ruffling chick heart fibroblasts (1.32 μm min⁻¹) as that obtained for arcs in this study. In addition, Abercrombie et al. commented that they observed 'a series of indefinite shadows chasing each other steadily backwards from the edge (of the lamella) in the region where particles move' and that these shadows, which I think were arcs, moved at speeds not widely different from that of particles.

So it is clear that the speed, the deceleration, and the distance travelled over the lamella are comparable for arcs and particles. And this was confirmed by the observation that, on a number of occasions, arcs and particles travel centripetally together on either side of the plasma membrane.

In conclusion, the structure of arcs and their association with particle transport raise some important questions about the mechanisms of centripetal flow. Do arcs move back through a stationary sheath of microfilaments or, as others have suggested (Abercrombie et al. 1970b; Harris, 1973; Dunn, 1980; Dembo & Harris, 1981), is the cell surface and/or the submembraneous layer of microfilaments moving back as a whole, and being assembled at the lamellar margin and disassembled near the nucleus? Furthermore, is the transport of particles directed by a transmembrane linkage with arcs? It is well known that cross-linked cell surface molecules can form transmembrane associations with actin and other components of the cytoskeleton (Sundqvist & Ehrnst, 1976; Ash, Louvard & Singer, 1977; Flanagan & Koch, 1978).

Although this study has not given many answers to these problems, it should be obvious that the behaviour of arcs offers, perhaps for the first time, a powerful experimental approach to understanding the mechanisms involved in the distribution and rearrangements of cell surface molecules and microfilaments in a moving cell.

I am very grateful to Dr Graham Dunn for his advice and constructive criticisms, and to Drs Stephen Paddock and John Couchman for help and discussion at various stages of the project.

Note added in proof: In a recent publication, Soranno & Bell (J. Cell Biol. 95, 127-136, 1982) have described similar arc-shaped microfilament bundles in spreading human fibroblasts.

REFERENCES

Centripetal movements in fibroblasts


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