THE BEHAVIOUR OF FIBROBLASTS
MIGRATING FROM CHICK HEART EXPLANTS:
CHANGES IN ADHESION, LOCOMOTION AND
GROWTH, AND IN THE DISTRIBUTION OF
ACTOMYOSIN AND FIBRONECTIN

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SUMMARY

Fibroblasts migrating from heart explants of chick embryos at first have a high rate of locomotion but lack focal contacts or adhesions and also lack substantial actin-containing bundles. A meshwork of 7-nm filaments is present particularly in submembranous regions and is proposed to be directed towards efficient locomotion whilst maintaining a high degree of spreading. Also during the first 48 h there is little production of extracellular fibronectin and the growth rate is low. Later, these fibroblasts develop focal contacts and focal adhesions together with actomyosin bundles, with a parallel increase in fibronectin expression. We propose that progressive immobilization by the development of focal adhesions and actomyosin structures occurs to set these cells up for growth.

INTRODUCTION

The development of a well-spread morphology in anchorage-dependent fibroblasts has often been correlated with the formation of actin-containing microfilament bundles (Ishikawa, Bischoff & Holtzer, 1969; Goldman et al. 1973; Lazarides & Weber, 1974). Conversely, transformed cells (McNutt, Culp & Black, 1971, 1973; Goldman, Chang & Williams, 1975; Weber et al. 1975; Pollack, Osborn & Weber, 1975; Ash, Vogt & Singer, 1976) and adhesion-defective mutants (Willingham et al. 1977) which have lost such bundles, also show a decreased degree of spreading. The development of microfilament bundles, or stress fibres, appears to be coupled to the formation of focal adhesions (Abercrombie, Heaysman & Pegrum, 1971; Heath & Dunn, 1978) which are organized structures in which a side-to-side array of external glycoproteins binds to substrate-absorbed fibronectin, to stabilize a sub-membranous complex to which the terminus of the bundle is anchored (Rees et al. 1978). We have found it useful (Couchman & Rees, 1979) to distinguish between such focal adhesions which are highly organized and long-lived structures giving a dense black image in the interference-reflexion microscope, and focal contacts which are similar in dimensions but are more transient and give a grey-black image.

Fibronectin (for a review, see Yamada & Olden, 1978) occurs in serum and is synthesized in a somewhat different form by fibroblasts in culture (Hynes, 1973;
The focal adhesions, or focal contacts, are characterized by an intimate contact of the lower cell membrane with the substratum (Abercrombie et al. 1971; Heath & Dunn, 1978; Rees et al. 1978) and most or all of the fibronectin at these sites (Badley et al. 1978) may be cell-derived (Rosen & Culp, 1977). Focal contacts can be visualized by interference-reflexion microscopy (Curtis, 1964; Abercrombie & Dunn, 1975; Izzard & Lochner, 1976) and Abercrombie, Dunn & Heath (1976, 1977) have demonstrated that they can have an important role in fibroblast locomotion. Rows of focal contacts form close to the leading edge and remain attached as the cell moves forwards over them. These authors propose that movement is produced by the exertion of tension in microfilament bundles extending from the focal contacts towards the nucleus and that as the nucleus approaches the contacts they disappear.

The detachment of fibroblasts from substrata by trypsin is preceded by changes in the structure of focal adhesions and then by the disintegration of stress fibres and rounding of the cell body (Pollack & Rifkin, 1975; Rees, Lloyd & Thom, 1977). The detachment and the changes to the stress fibres can be blocked by pretreatment of cells with Con A (Rees et al. 1977), which also limits the visible changes in the focal adhesions. The lectin is believed to act by constraining the lateral movement of surface receptors.

In a preliminary communication (Couchman & Rees, 1979) we have described a population of chick heart fibroblasts, prepared without the use of detachment agents, having different morphological characteristics to cells adapted to growth in culture. These fibroblasts are highly locomotory, but lack focal adhesions or focal contacts yet are nevertheless very well spread and have good adhesion characteristics. These motile cells also have a low growth rate. We now describe a more detailed investigation of these cells and also their subsequent changes in morphology resulting in the development of focal adhesions together with more substantial actin-containing microfilament bundles, and a reduction in motility. Once in this state the cells have a much higher growth rate but are as well spread as the initial cells. Fibronectin production is also greatly increased at this time. We conclude that the development of substantial microfilament bundles and focal adhesions or contacts is not necessarily required for the development of adhesion and spreading, or indeed for locomotion. There does, however, seem to be some correlation between the development of adhesions and microfilament bundles with growth and reduction of movement.

**MATERIALS AND METHODS**

**Cells and light microscopy**

Chick heart ventricle from 7—9-day-old embryos was explanted into a small chamber bounded by sterile glass coverslips containing 40 µl Modified Basal Eagle's Medium (MBEM) with Hanks' salts, supplemented with 10% foetal calf serum (FCS) (Flow Laboratories, Irvine, Scotland). Phase-contrast and interference-reflexion microscopy were performed on live cells maintained at 37 °C by a thermostatically controlled fan heater. Photographs were taken on a Leitz Ortholux microscope fitted with epi-illumination and phase-contrast, interference-contrast and interference-reflexion objectives (Leitz) with Ilford FP4 film.
Time-lapse video recordings were made on a National Panasonic VTR (NV-8030) with a Hitachi CCTV camera and monitor (HV-620K and VM-126AK respectively). Estimates of mitosis rates were derived from time-lapse video recordings repeated several times over the first 72 h after cell emergence. On each occasion fields of 200–300 cells were filmed using phase-contrast optics.

**Electron microscopy**

Cells were fixed in 3 % glutaraldehyde in 0·1 M sodium cacodylate buffer (pH 7·0) for 30 min, washed in buffer then postfixed in 1 % osmium tetroxide in the same buffer for a further 30 min. Pre-treatment was achieved by a 30-min treatment with 1 % aqueous uranyl acetate, and the cells were then dehydrated through a graded ethanol series, transferred to propylene oxide and embedded in Araldite resin. Thin sections were cut on a Porter-Blum ultramicrotome and viewed on a Jeol 100C electron microscope. For scanning electron microscopy cells were fixed in 3 % glutaraldehyde in 0·1 M sodium cacodylate buffer (pH 7·4) for 1 h, washed in buffer and dehydrated through a graded ethanol series, and critical point dried through carbon dioxide in a Polaron critical point bomb. Coverslips were mounted on brass specimen stubs using colloidal silver and coated with ~10 nm of gold in a Polaron E5100 ‘cool’ sputter-coating unit. The cells were examined in a Jeol JSM-35X electron microscope at a 25-kV accelerating voltage.

**Quantimet image analysis of cell areas**

Chick fibroblasts prepared as above were viewed with phase-contrast optics on a Leitz Ortholux II microscope connected to a Quantimet 720 (Cambridge Instruments). An image editor was used to define spread cell perimeters and the Quantimet was interfaced to a D.E.C. PDP 11/05 to give calibrated cell areas.

Data were prepared for spread cells with and without focal adhesions. Groups of results were compared by analysis of variance to establish whether the populations had statistically significant differences in cell area.

**Autoradiography**

Chick heart explants were prepared and incubated in plastic Leighton tubes lined with glass coverslips for 24 h at 37 °C in MBEM with Hanks’ salts and 10 % FCS. The coverslips were twice washed with Hanks’ balanced salts solution (HBSS) (Flow Laboratories) and the coverslips divided into 2 groups. One group (controls) was incubated with HBSS for 10 min at 37 °C then washed with complete MBEM. The other group was incubated with 5 μg/ml trypsin (type III, Sigma Chemical Co., Parkstone, Dorset) in HBSS for 10 min at 37 °C then washed with complete MBEM.

All samples were then incubated with complete MBEM containing 1 μCi/ml tritiated thymidine (TRA.120, Radiochemical Centre Ltd., Amersham, Bucks.) in an atmosphere of 10 % CO₂ in air at 37 °C. At 2-h intervals control and trypsinized samples were fixed in 3·5 % formaldehyde in phosphate-buffered saline (PBS) (Gibco, Paisley) for 30 min then washed in 0·1 % ammonium chloride in PBS. The coverslips were immersed for a few seconds in a solution containing equal volumes of 1 % glycerol and K5 photographic emulsion (Ilford). After drying they were kept at 4 °C for 7 days then processed for 3 min in D19 developer (Kodak), washed, then treated for a further 3 min in 50 % hypo. After prolonged washing the coverslips were then mounted and examined. 400 cells were counted from each coverslip and the percentage having grains over the nucleus calculated.

**Indirect immunofluorescence microscopy**

The distribution of actin-containing structures and extracellular fibronectin was studied by indirect immunofluorescence microscopy. Antibodies to smooth muscle actin and chick plasma fibronectin were provided by Drs R. A. Badley and D. Thom respectively. The method of preparation of these antisera and their use in staining of cells has been fully documented elsewhere (Badley et al. 1978; Thom, Powell & Rcs, 1979). A fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antiserum (Nordic Diagnostics, London) was used in both cases.
Photographs were taken on a Leitz Ortholux II microscope fitted with epi-illumination on Ilford HP5 film.

**Detachment studies**

Cells prepared as above were washed with calcium- and magnesium-free HBSS and treated with 10 µg/ml trypsin (Sigma) in calcium- and magnesium-free HBSS at 37 °C. In some studies cells were pretreated for 20 min at 37 °C with 20 µg/ml Con A (Pharmacia (G.B.) Ltd, London) in HBSS, followed by 2 washes in HBSS. These cells were then treated with trypsin as above.

**RESULTS**

**Morphology of the chick heart fibroblasts**

The first cells to emerge from a heart explant were well spread (Fig. 1) and locomotory, rates up to 80 µm/h being recorded from numerous time-lapse video recordings. These cells generally had a wide but very thin leading edge (Fig. 1) and were characterized by their extreme degree of spreading. The image of these cells seen by interference-reflexion microscopy continuously fluctuated but was predominantly

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Fig. 1. Interference-contrast micrograph of a typical initial migratory fibroblast, with a well-spread morphology and broad, thin leading edge (arrowhead).

Fig. 2. Interference-reflexion micrograph of an initial migratory fibroblast showing a mottled image, mostly of shades of grey. There are no structures corresponding to focal contacts or adhesions.

Fig. 3. Initial migratory fibroblast with transient small dark patches in the interference-reflexion image (arrowhead) which do not correspond to focal contacts.

Fig. 4. Interference-reflexion micrograph of a later chick fibroblast clearly showing numerous focal contacts in the leading edge.
shades of dark grey, indicating a very close association with the substratum (Fig. 2). The central area of the cell was frequently mottled with lighter patches but no structures corresponding to focal contacts or focal adhesions could be seen, though some cells had a trailing extremity which appeared black at its tip by interference-reflexion microscopy. Small zones which were darker than adjacent areas were sometimes seen by this technique (Fig. 3), appearing in any area of the cell, but within a few minutes they became lighter and merged imperceptibly with the image of the rest of the cell. Some cells could be seen by interference-reflexion microscopy to have bands of a uniform nature which were oriented parallel to the leading edge and which appeared to pass backwards as the cell moved forwards. Whilst moving cells showed sensitivity to cell-cell contact in that they would change direction as a result of collision, there was not a strong contact-retraction response.

Within 48 h some of the fibroblasts started to form small discrete black areas as seen by interference-reflexion microscopy, corresponding to focal contacts. These tended to form close to the leading edge and were oriented with the longer axis of the contact parallel or nearly parallel to the direction of movement and perpendicular to the margin of the leading edge (Fig. 4). Some focal contacts were capable of rapid

Figs. 5, 6. Interference-reflexion micrographs of the leading edge of a chick fibroblast with a 5-min interval between photographs. The 2 focal contacts (arrowhead) have disappeared without significant forward locomotion having taken place.

Fig. 7. Stationary fibroblast with numerous focal adhesions showing black in the interference-reflexion image. The position of microfilament bundles running close to the substratum can be seen (arrowhead).

Fig. 8. Phase-contrast micrograph of the same cell as Fig. 7 showing the well-spread nature of the cell, with a more polygonal morphology than initial migratory cells.
dissolution without any substantial locomotion having taken place (Figs. 5, 6). Fibroblasts with arrays of focal contacts were capable of comparable rates of locomotion to those lacking them, and the outline shape of these cells was similar, both types being well spread. When the frequency of cell-cell contact increased as a result of increasing numbers of cells migrating from the explant, cell locomotory activity became markedly reduced. In parallel with the reduction of locomotion, large numbers of fibroblasts changed from a 'fan-shape' typical of moving cells, to a more polygonal morphology. These altered cells had a rather different image as seen by interference-reflexion microscopy (Fig. 7), with large areas of the cell being of pale grey to white colour, indicating a greater separation from the substrate than previously. Areas corresponding to focal adhesions showed as distinct black areas usually located towards the cell margins and particularly at the apices. Focal adhesions were rather larger than the structures described above as focal contacts and were more permanent in nature. These cells were also very well spread (Fig. 8) and statistical analysis of their areas compared with initial moving fibroblasts shows no significant difference as measured by Quantimet Image Analysis (Table 1). Fig. 9 shows a schematic representation of the stages through which the cells pass.

**Table 1. Quantimet image analysis of cell areas**

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean area migratory cells, ( \mu m^2 \pm S.D. )</th>
<th>Mean area stationary cells, ( \mu m^2 \pm S.D. )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>1170 ± 287</td>
<td>964 ± 218</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>843 ± 211</td>
<td>709 ± 148</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>1178 ± 227</td>
<td>1031 ± 311</td>
<td>&gt; 0.5</td>
</tr>
</tbody>
</table>

From 48 h after explantation onwards, increasing numbers of cells cease or reduce locomotory activity and form focal adhesions, including many of those cells which were at the perimeter of the fibroblast population and not therefore restricted from movement by contact inhibition. Where cell movement did occur it was frequently much slower than 80 \( \mu m/h \) and cell–cell contacts resulted in strong contact-retraction behaviour. The pattern of morphological change reported here was always observed provided that the cells had not been exposed to detachment agents. In several experiments (Couchman & Rees, unpublished) in which tissue was first dissociated by trypsin, we found that the outwandering cells were nearly all characterized by focal contacts or adhesions. In these circumstances the movement observed and the pattern of focal contact formation was similar to that described by Abercrombie et al. (1976, 1977). It would appear that proteolytic action alters the sequence of events, and we have found that addition of trypsin at later stages of development also enhances focal adhesion formation.

**Growth rates and cell cycle**

The rate of growth of explant-derived chick fibroblasts was strongly correlated with the development of focal adhesions. In the first 48–72 h after explantation the cells
had an extremely low growth rate, in the order of 7% of the cells per 24 h. A population of chick fibroblasts adapted to culture had, under similar conditions, a rate of mitosis of very nearly 100% of the cells per 24 h. To establish the stage in the cell cycle of the migratory fibroblasts with low growth rate, they were stimulated with 5 μg/ml trypsin, then exposed to [3H]thymidine. As shown in Fig. 10, mild trypsin treatment stimulated the cells to incorporate thymidine more rapidly than in a control in which the medium had been changed for trypsin-free medium. A change of medium was itself noted to have a mild stimulatory effect (Couchman & Rees, in preparation).
Incorporation of thymidine by trypsin-treated cells occurred in advance of any significant increase in mitosis rate observed by filming, which began at 8–10 h after stimulation. The migrating cells were, therefore, in the $G_1$ phase of the cell cycle.

**Extracellular fibronectin distribution**

The production and expression of fibronectin by the chick heart fibroblasts was monitored from the beginning of migration for a period of 72 h. Migrating fibroblasts expressed very little fibronectin on their surfaces that could be detected by indirect immunofluorescence (Fig. 11), but the amount increased after several cell–cell contacts had been made. By 72 h large quantities of fibronectin could be demonstrated on interphase non-migratory cells (Fig. 12), mostly over the upper surface, but some

![Fig. 11](image.png)

**Fig. 11.** A group of migratory fibroblasts from a 24-h-old culture stained for fibronectin by an indirect immunofluorescence technique showing small amounts, mostly in cell–cell contact areas.

![Fig. 12](image.png)

**Fig. 12.** Chick fibroblasts from a 72-h-old culture containing mostly stationary cells stained for fibronectin as in the previous figure. Here fibronectin is present in large quantities as a cell surface glycoprotein forming a pericellular matrix.

extending between and under the cells. The fibronectin was nearly all in the form of fibrils, which often extended for many micrometres.

**Electron microscopy and actomyosin distribution**

We have described the initial migratory fibroblasts as lacking focal contacts or adhesions, and this is supported by evidence from transmission electron microscopy. Fig. 13 shows large areas of unspecialized membrane in very close association with
the substratum, with a lack of areas characteristic of focal contacts or adhesions; the
distance of separation from the substratum was mostly less than 30 nm but variable.
Interestingly, these cells also showed a lack of conspicuous microfilament bundles,
though a submembranous meshwork of 7-nm filaments of variable density was
present close to the upper and lower cell membranes (Fig. 13). Meshwork of micro-
filaments was also found in more internal regions of the cells, particularly in perinuclear
locations, and this was generally much less dense than that in submembranous areas.
The submembranous meshwork was shown to better advantage by horizontal thin
sections parallel to the substratum (Fig. 14). A broad band of 7-nm filaments could
be seen traversed by a few thin bundles oriented parallel to the direction of loco-
motion.

Where focal contacts and adhesions were present in later stages, these could be
seen in vertical section (Fig. 15) together with conspicuous microfilament bundles.
The more substantial nature of these bundles compared with initial migratory cells
was often seen by scanning electron microscopy. Fig. 16 shows the leading edge of
an initial moving fibroblast in which microfilament bundles could not be detected,
whilst their position could be clearly seen in later fibroblasts (Fig. 17).

Further information regarding the distribution of actin-containing structures was
obtained by indirect immunofluorescence microscopy. In early migratory cells (Fig.
18) bundles of microfilaments are thin and few in number, though usually oriented
parallel to the direction of movement. Diffuse staining was also seen and taken to
originate from the microfilamentous meshwork seen by electron microscopy. Such
diffuse staining was reduced in stationary cells having focal adhesions where micro-
filament bundles were generally more substantial and numerous (Fig. 19).

Adhesion and detachment studies

We have already shown (Couchman & Rees, 1979) that the nature of adhesion
between the cell and substratum differed between the initial migratory fibroblasts
lacking focal contacts or adhesions and cells adapted for culture which had these
structures. Nevertheless both types of cell were shown to adhere strongly to the
substratum. In contrast to the behaviour of cells having focal adhesions which readily
rounded up in 10 μg/ml trypsin and at intermediate stages left retraction fibrils
between the cell body and points of initial strong attachment to the substratum, moving
chick fibroblasts were less susceptible. They detached less readily and when rounding
up left few or no retraction fibrils to the substratum suggesting a different cell/sub-
stratum relationship (Couchman & Rees, 1979).

Previous work (Rees et al. 1977) has shown that fibroblasts adapted to grow in
culture can be protected against trypsin detachment by pretreatment with Con A.
We find that, in contrast, the moving cells are not thus protected by 20-min pre-
treatment with 20 μg/ml Con A at 37 °C; indeed, subsequent exposure to 10 μg/ml
trypsin caused rapid detachment which was 80% complete within 5 min. Chick
fibroblasts seen to have focal adhesions by interference-reflexion microscopy do not
detach from the substratum when treated with the same concentrations of Con A
then trypsin at 37 °C; they show some contraction after the pretreatment with Con A
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Fig. 16. Scanning electron micrograph of a migratory fibroblast lacking focal contacts. No microfilament bundles can be discerned.

Fig. 17. Scanning electron micrograph of a migratory fibroblast of a later stage. The positions of microfilament bundles can be clearly seen (arrowheads).

Fig. 18. Indirect immunofluorescence visualization of actin in an initial migratory fibroblast. Diffuse staining of the meshwork is predominant.

Fig. 19. Indirect immunofluorescence visualization of actin in a stationary fibroblast showing large numbers of microfilament bundles and a decrease in diffuse staining.

Fig. 13. Transmission electron micrograph of a vertical section of an initial migratory fibroblast showing a uniform unspecialized lower cell membrane and a meshwork of 7-nm filaments within the cytoplasm (arrowhead), particularly in a submembranous location, where they form a more dense network.

Fig. 14. Transmission electron micrograph of a horizontal section of an initial migratory fibroblast showing large amounts of microfilamentous meshwork in a submembranous distribution. A thin microfilament bundle is also present (arrowhead).

Fig. 15. Transmission electron micrograph of a focal contact in a stationary chick fibroblast, with an associated microfilament bundle.
and cell rounding is only marginally increased by post-treatment with trypsin. These results demonstrate the different nature of the adhesion between cells which are migratory and lack membrane specialization in the form of adhesive foci and those cells which are non-migratory, having focal adhesions.

DISCUSSION

The results presented above show that chick heart fibroblasts migrating from explant tissue progress through a sequence of stages culminating in a virtually non-migratory state where adhesion to the substrate is through focal adhesions surrounded by areas of membrane with a greater separation from the substrate. In parallel there is an increase in the number and thickness of actin-containing microfilament bundles which is consistent with previous evidence that the development of these structures is correlated with that of the focal adhesions (Abercrombie et al. 1971; Heath & Dunn, 1978; Rees et al. 1978). The first fibroblasts migrating from an explant while lacking focal contacts or focal adhesions, are highly locomotory with large areas of unspecialized membrane closely associated with substratum. This correlates well with the suggestions of Abercrombie & Dunn (1975) and Izzard & Lochner (1976) that close contacts can be involved in cell locomotion. From our electron-microscope studies, however, it appears that the areas of unspecialized cell membrane may be separated by distances less than 30 nm from the substrate, indeed approaching 10-14 nm, which has been proposed as the separation distance at focal contacts (Izzard & Lochner, 1976). The darker image of focal adhesions in the interference-reflexion microscope might therefore result from the increased specialization and accumulation of cellular and extracellular components at these structures leading to an increase in refractive index, rather than to an altered separation distance between the cell membrane and substratum.

The first migrating fibroblasts had a substantial part of their microfilament component in the form of a meshwork which is less obviously structured than that in stress fibres and seems to have a dynamic function expressed as cell movement. After 24-48 h focal contacts appeared which we distinguish from focal adhesions by their smaller size in the interference-reflexion image and also by their transience, since they can disappear rapidly. At this stage we have seen the type of cell movement described in the classical studies of Abercrombie et al. (1976, 1977). Even here, however, some part of the locomotory process might continue to be driven by a meshwork of actin-containing microfilaments, especially the protrusive activity at the leading edge which requires the forward movement of material (Abercrombie et al. 1976) and which is difficult to imagine being generated by the microfilament bundles themselves. The fan-shaped morphology of motile cells shows that actomyosin is directed to protrusion rather than, as later, withdrawal. We suggest that this activity is driven by the microfilamentous meshwork since it has been shown here and elsewhere to be present in sub-membranous locations (Ishikawa et al. 1969; Lazarides & Weber, 1974).

Another system in which actin bundle formation correlates inversely with cell locomotion has been demonstrated by Wehland, Osborn & Weber (1977) and Wehland,
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Stockem & Weber (1978). Cultured fibroblasts and *Amoeba proteus* develop an extensive array of actin-containing bundles when injected with low levels of phalloidin, a bicyclic polypeptide isolated from the fungus *Amanita phalloides*. Following this bundle promotion there is a complete cessation of locomotion.

The lack of specialized structures between the lower membrane of migratory cells and substratum is very similar to that of macrophages which do not show adhesive plaques under interference-reflexion microscopy (Abercrombie et al. 1977), but which do possess large amounts of submembranous microfilament meshwork (Allison, Davies & de Petris, 1971; Reaven & Axline, 1973). Macrophages under usual culture conditions do not divide (Page, Davies & Allison, 1978), and this is another property in common with our early migrating fibroblasts. Our results indicate that the development of microfilament bundles, whilst not being essential for movement might contribute to other cellular functions such as those essential for growth. These results can be compared with the finding (Folkman & Moscona, 1978) where a degree of cell flattening is required for [3H]thymidine to be incorporated in certain nontransformed cultured fibroblasts. In our system, spreading alone is clearly insufficient to set up the conditions for growth because no increase in spreading is seen in the transition from early locomotory fibroblasts to later dividing cells. Rather, a conversion occurs to a particular type of spreading which is associated with focal adhesions and microfilament bundles. Others, however (Willingham et al. 1977), have shown for several cultured fibroblast lines that the generation time is not necessarily shortened when culture conditions are altered to convert the cell from a rounded morphology in which stress fibres are not visible by immunofluorescence to a flattened form with conspicuous fibres, although some instances were noted in which this did occur. All this suggests to us that the anchorage requirement for growth could be for a degree of cell spreading which depends on the cell genotype, to commit a proportion of cellular actin to a stable relationship with adhesion areas. In our system which may be analogous to a wound response, the chick fibroblasts are initially specialized for locomotion with adhesion and spreading being associated with a dynamic form of actomyosin which is independent of focal adhesions, and cell division is suspended. Although at first sight surprising, the outgrowth of cells in other experimental systems have yielded similar results, in that initial migration also occurs without growth (Gibbins, 1976; Shelley, Gimborne & Cotran, 1977; Stenn, 1978). We have shown that the migratory fibroblasts are in $G_1$ phase of the cell cycle and progress through $S$ when a stationary stage has been achieved, or when stimulated experimentally by mild trypsin treatment. Exposure to trypsin leads to the promotion of focal adhesion formation in our system, and we have also described these structures in stationary fibroblasts which leads us to conclude that the attainment of stability through focal adhesions and their associated microfilament bundles may be an important prerequisite for these cells to progress through $S$, and subsequently, mitosis.

Despite the lack of focal contacts, the early migrating cells did show some contact inhibition of movement and subsequent redirection of movement after cell–cell contacts were made. In fibroblasts which show contact retraction more strongly, it has been established (Abercrombie, 1970; Abercrombie & Ambrose, 1958; Aber-
crombie & Dunn, 1975) that cell–cell contact quickly leads to localized adhesion formation between the cells (Heaysman & Pegrum, 1973) before the retraction response. Since the initial migratory cells lack focal contacts or adhesions with substratum, their limited contact retraction response is perhaps understandable in terms of similar inability to form cell–cell adhesions. Later, when the cells have adhesions to substratum, their contact retraction is stronger.

The strength of adhesion to substratum seems to be similar in the moving fibroblasts and the non-migratory fibroblasts, whether this is measured by the susceptibility to detachment agents or to mechanical dislodgement (Couchman & Rees, 1979). However, the differences in the patterns of contacts with substratum, in the organization of cellular actomyosin, and in the effect of pretreatment with Con A on the rate of detachment by trypsin, all indicate that the mechanisms of adhesion must be different. We have proposed (Rees et al. 1977) a distinction between contributions to cell adhesion from forces acting at the outer surface — which we called ‘stick’ — and those from internal structures which we called ‘grip’. Grip can augment adhesion (i) by presenting surface macromolecules to substratum in an appropriate configuration, (ii) by flattening the cell to present a lower profile to shear and turbulence in the medium, (iii) by stiffening the cell so that it is peeled away less readily, and (iv) by stabilizing the cell shape to diminish the elastic energy that would otherwise be stored from the deformation for efficient contact. In fibroblasts adapted to culture, these effects are achieved through relatively limited but highly organized (Rees et al. 1978) and stable contacts with substratum at the focal adhesions, with the associated stress fibres enhancing the various contributions from cell shape. In the moving fibroblasts, a greater area of contact with substratum seems to compensate for the lack of the local organization and intimacy of contact in focal adhesions, and the cell shape contributions are from a more dynamic form of actomyosin. Presumably these 2 different mechanisms have functional advantages for the growth phase and the motile phase of the cells, respectively.

Fibronectin was expressed in very small amounts on the cell surface of the initial migratory heart fibroblasts, but as cell–cell contacts increased the amount of fibronectin that could be visualized by indirect immunofluorescence microscopy also increased. In parallel with an increase in cell surface fibronectin was a progressive immobilization of the cells, resulting from the formation of focal adhesions and stress fibres. All this is consistent with the observations of others, that fibronectin expression is related to cell density (Hynes & Bye, 1974), and is promoted by cell–cell contact (Chen, Moser, Chen & Mosesson, 1977; Chen et al. 1978). Fibronectin has also been shown to be a component of the focal adhesion (Rees et al. 1978; Badley et al. 1978) and has been shown to promote stress fibre formation (Yamada, Yamada & Pastan, 1976; Yamada, Ohanian & Pastan, 1976; Willingham et al. 1977; Ali, Mautner, Lanza & Hynes, 1977; Chen et al. 1978; Yamada, Olden & Pastan, 1978).

In conclusion, we have described a population of chick embryo fibroblasts which on emergence from a heart explant are highly migratory, possess few microfilament bundles, express little fibronectin and lack focal contacts or focal adhesions. They also have a low growth rate. These observations demonstrate that cellular activity is
directed towards a locomotory phase where cellular actin is less visibly structured but is highly dynamically organized for movement whilst maintaining good spreading and adhesion characteristics. Later stages show a decrease in locomotion together with formation of focal contacts and adhesions, which, with a concomitant production of stress fibres results in the commitment of actin into structures which stabilize the cell for growth.

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