THE RELATIONSHIP OF FIBROBLAST TRANSLOCATIONS TO CELL MORPHOLOGY AND STRESS FIBRE DENSITY

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SUMMARY

Translocation of human fibroblasts in culture was studied using techniques of time-lapse cinemicrography, indirect immunofluorescence, and computer analysis. An inverse relationship between the velocity of cells during the last hour of life and the density of stress fibers seen by immune staining was demonstrated. Translocating cells generally assumed one of two interconvertible morphologies: a triangular tailed shape or tailed fibroblast (TF), and a tailless form that resembled a half-moon, which we call a half-moon fibroblast (HMF). The tail of TFs formed only on regions of substrate that had been previously traversed by cells. The half-moon morphology developed either on previously used or on virgin substrate. Cells adopted the HMF rather than the TF morphology with a four-fold greater frequency. HMFs translocated slightly faster than TFs. The foregoing observations suggest that the fibroblast tail is not an organelle essential for translocation. Since our technique allowed us to distinguish between cells which were cycling and those which had left cycle, we compared their velocities and found them to be similar. Also the average velocities of cells of different population-doubling levels (10th, 30th and 40th) were approximately equal.

INTRODUCTION

The motility of fibroblasts has been studied mainly on glass or plastic substrates, both different from the three-dimensional tissue world which fibroblasts normally inhabit. Based on observations of cells locomoting on two-dimensional substrates, a view has emerged that stress fibres may play an important role in fibroblast translocation. In fact, this view has developed in part from an idealized conception of fibroblast morphology. The cell has been pictured roughly as an isosceles triangle whose leading edge is the base and whose trailing edge is a small-angled apex (Ambrose, 1961). Two main sets of stress fibres are described: one, radiating forward toward the leading edge from an anterior perinuclear zone (Abercrombie, Heaysman & Pegrum, 1970; Abercrombie, Dunn & Heath, 1977), and the other converging at the cell apex from the posterior perinuclear zone (Goldman, Schloss & Starger, 1976). By stress fibres, we mean the bundles of 6-7 μm filaments originally described by Buckley & Porter (1967) and seen by them with phase microscopy. The fibres consist of actin complexed with other mechanochemical proteins (Weihing, 1979). From

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experiments carried out with isolated stress fibres, it is clear that they possess contractile properties (Isenberg et al. 1976). In a recent paper written shortly before his death (the 1978 Croonian Lecture), Abercrombie (1980) presented a theoretical view of the contribution of anterior stress fibres to fibroblast translocation. After reviewing evidence that stress fibres terminate anteriorly in focal contacts (Heath & Dunn, 1978), the structures by which a fibroblast appears to be attached to the substrate, Abercrombie suggested that contraction of fibres pulls the nuclear region or the main mass of the cell forward. As this occurs, the leading edge of the cell extends, new focal contacts are established, and these in turn develop stress fibres. Lamellipodial protrusion and stress fibre contraction, mainly, are held responsible for cell translocation. The continued forward movement of the cell is accompanied by release of the tail attachments from the substrate. As the fibroblast advances, if a tail is present, it undergoes stretching and eventually snaps forward. The snap and shortening could be due to elastic recoil after the stretch, or to contraction of the stress fibres which lie posterior to the nucleus and terminate in the cell apex. Some workers hold that tail snapping is the result of stress fibres contracting and suggest that the phenomenon of tail shortening accounts for ‘much of the net translocation of fibroblastic cells’ (Goldman et al. 1976). Chen (1979) considers tail retraction a necessary event for the normal protrusive activity at the leading edge of a cell. An hypothesis put forth by Albrecht-Buehler (1978) also implicates stress fibres as part of the machinery of motility, suggesting that the main mass of the cell slides on stress fibres as a train moves over rails.

An alternative view correlates the presence of stress fibres with immobile cells and their sparseness or absence with motile cells (Fujiwara & Pollard, 1976; Couchman & Rees, 1979; and Badly, Couchman & Rees, 1980).

The results of the present study tend to confirm and extend the latter view. In the course of studying the translocation of large numbers of AG 1519 human fibroblasts in computer-assisted cell lineage studies Bell et al. (1978, 1979b and unpublished results) observed that many, if not most, motile fibroblasts were not rough isosceles triangles but were tailless cells shaped like half-moons. These observations stimulated us to compare the motility of fibroblasts lacking tails with that of the classical tailed fibroblast.

In this study, we have correlated the velocity history of particular cells just before fixation with their morphology and their stress fibre patterns visualized through immune staining, immediately after fixation, and present evidence that motile cells are stress-fibre poor while stationary cells are richly endowed with them. We find that fibroblasts need not have tails to be motile; in fact, that the tailless morphology of AG 1519 fibroblasts is assumed more frequently than the tailed form. We also observed that tails only form on regions of the substrate that have been previously travelled by one or more cells. We show in greater detail than before (Bell et al. 1978) that the cycling state of cells has little to do with their locomotory capacity. Cells in and out of cycle and of widely different population doubling levels, when they are moving, travel with similar velocities.
Fibroblast translocations

METHODS AND MATERIALS

Cell cultures

Stock cultures of human foreskin fibroblasts (AG 1519) were grown in McCoy's 5A medium with 20% foetal calf serum at 37 °C in a 5% CO₂ atmosphere. The culture medium also contained 1 unit/ml of penicillin, 1 unit/ml of streptomycin, and 2.5 µg/ml Fungizone. Cells of the 10th, 30th and 40th population doubling levels (PDLs) were studied. Cell cultures studied were plated at different densities onto 2 in. diameter Gold coverslips. The coverslips were cleaned by rinsing in 100% ethanol, wiped dry, and then sterilized in an autoclave. After the cells had settled overnight, nine coverslips were placed in each Cooper Dish (Falcon brand, 60 x 15 mm). Each dish contained coverslips of different cell densities. We added 5-5 ml of culture medium to the dish which was sealed with sterile silicon stopcock grease laid in the angle of the cover.

Cinematographic records

Coverglasses in each Cooper dish were surveyed to select for one with few cells and its location was noted. The Cooper dish was positioned on an inverted Nikon microscope (model M) equipped with a 2.5 x (NA = 0.08) or 4 x (NA = 0.16) plan apochromatic objective lens (Zeiss) modified for Hoffman modulation contrast microscopy (Hoffman, 1977). Filming was carried out in a 37 °C room where cells were provided with a 5% CO₂ atmosphere by flooding a plastic bag draped over the microscope. A time-lapse system previously described (Bell et al. 1979b) was used. The film employed was Kodak technical pan 16 mm film ESTAR Base SO-115. The starting frame of each film contained no more than a few cells, one of which seemed ready for division. One frame was taken every one or five minutes for three to eight days.

Indirect immunofluorescence

Immediately after filming, coverslips were placed in 3.7% formaldehyde in phosphate buffered saline (PBS) for approximately 30 minutes. The coverslips were then rinsed in acetone and then PBS. Next, each coverslip was incubated for 60 min at 37 °C with antisera to actin or myosin. Antibodies to actin were raised in rabbits after they were injected with chicken gizzard actin (Spudich & Watt, 1971). In addition to antibodies to actin prepared in our laboratory, we used an antiactin preparation which was a gift of Dr K. Burridge and an antymyosin preparation which was a gift of Dr R. Goldman. Each coverslip was then washed with PBS and incubated with goat anti-rabbit gammaglobulin conjugated to fluorescein (Cappell Laboratories, Inc.). Cells recorded in the final frame of the film were located on the coverglass and photographed with a standard Zeiss epifluorescence microscope using a 40 x (NA = 1) or 63 x (NA = 1.4) planapochromatic objective lens and blue excitation FITC illumination. Kodak Tri-X pan (ASA 4000) film was used and developed to an ASA of 1600 with Diafine.

In order to determine the relationship between the presence of stress fibres and cell velocity, photographs of fluorescent images were examined and cells were scored by three persons who had no knowledge of the velocities of the cells, as belonging to 1 of 3 classes based on stress fibre density. Class 1 included cells with few or no stress fibres. Class 3 included cells with many stress fibres and Class 2 included intermediate examples. There were no conflicting judgements.

Film analysis with the interactive computer system

Cell coordinate data are gathered with an interactive system modified after Bell et al. (1972b) in which an operator moves a cursor by hand to follow a cell in a time-lapse film projected on a screen by a computer-controlled motion picture projector. The cursor is positioned over the cell nucleus. One (x, y) point corresponding to the position of a cell in each frame is recorded. The tablet resolution is 0.25 mm on the screen which corresponds to 1.3-1.6 µm on the microscope slide depending upon the optics used.

A computer program was written for the purpose of calculating short-time velocities of translocating cells. The user specifies two time points given by frame numbers and the program produces an estimate of the length of the trajectory executed by the cell. This path length
may be interpreted as a time-average velocity when divided by the time interval (Levinstone, 1981).

For comparison of velocities of half-moon fibroblasts (HMFs) and the tailed fibroblasts (TFs) cell trajectories which met two requirements were selected: (1) they were executed by cells which made no contacts with other cells in the course of their excursions, (2) trajectories studied were of translocations which lasted 1-6 h, long enough to observe a displacement. A TF was considered as such only if its tail snapped or broke off after a translocation; that is, if a cell formed a structure that morphologically resembled a tail, but it did not snap or break, it was excluded from our data.

Because the trajectory data included represented the movements of a cell as a series of points, the width of the trajectory was not recorded. When it was necessary to know if the substrate had been previously travelled by a cell, a piece of translucent paper was placed over the projection screen and the swath of substrate used by the cell was traced. Since we began essentially with a single founder cell on a virgin substrate, we were reasonably certain that tracks were not present before filming began.

RESULTS

Relationship between the presence of stress fibres and cell velocity

A total of 62 cells from four films were selected for study. Selection was made after unequivocal identification on the coverglass after staining of cells pictured in the final frame of the film. Only those cells which were out of contact with other cells, or in minimal transient contact with other cells were studied. Cells of the following population doubling levels (PDLs) were subjects: 9th, 15th, 30th and 50th. Cells were stained for actin to make stress fibres visible.

As defined previously, a typical Class 1 cell contained few or no stress fibres when stained for actin (Fig. 1). A Class 3 cell contained many stress fibres when stained for actin (Fig. 2). Cells with an intermediate number of stress fibres were scored as Class 2. The average velocity of Class 1 fibroblasts was 2.8 times greater than that of Class 3 cells and 1.8 times greater than that of Class 2. While over 50% of Class 1 cells translocated with a velocity equal to, or greater than 1.0 μm/min, only 4% of the Class 3 cells had such high velocities (Fig. 3). In fact, 69% of the Class 3 cells moved more slowly than 0.5 μm/min (Fig. 3). For the last hour of their lives, the average velocity of Class 1 cells was 1.1 ± 0.3 μm/min. Qualitatively, results similar to those observed when cells were stained with antibodies against actin, were seen when cells were stained with antimyosin antibodies. The density of stress fibres in cells stained for myosin was compared with cell velocity in each of seven cells identified in the final frame of a film in which the founder cell was of the 30th PDL. Three cells were of Class 1 and had average velocities of 1.7, 1.5 and 0.2 μm/min during the final hour of life while four were of Class 3 with velocities of 0.4, 0.2, 0.1 and 0.0 μm/min (see Figs. 4 and 5 for examples). It appears, then, that the presence of stress fibres is inversely related to cell velocity.

No relationship was observed between PDL and the presence of actin staining stress fibres. We noted 10 of 24 cells of the 10th PDL belonged to Class 1 compared with 2 of 21 cells of the 30th PDL and 9 of 19 cells of the 40th PDL. In addition, 7 of 24 cells of the 10th PDL belonged to Class 2 compared with 1 of 21 cells of the 30th PDL and 7 of 19 of the 40th PDL. Finally, 7 of 24 cells of the 10th PDL belonged
to Class 3 compared with 18 of 21 cells of the 30th PDL and 3 of 19 of the 40th PDL. Neither was there a relationship between the age of a cell after birth and the presence of stress fibres. For example, consider two cells that were the products of the same cell division 6 h before the end of a film and fixation. After staining it was observed that one cell had no stress fibres while the other had a distinct meshwork. Another cell 5.9 days old had thick stress fibres, a cell that was 7.3 days old had relatively few stress fibres and a 13.5 day old cell had thick bundles. The data presented are consistent with the view that the presence or absence of stress fibres is related to the motile state of a cell and not to PDL, and amplify previous findings of intraclonal heterogeneity already reported (Bell et al. 1978, 1979a, b).

Fig. 1. A typical Class 1 cell has few stress fibres and a high velocity. The arrow indicates the direction of translocation. The nuclear region stains brightly and diffusely. The edge of the plasma membrane, especially at the leading edge, also stains well. Stress fibres are thin and often perpendicular to the direction of movement (arrow). The stain was a fluorescein conjugated anti-actin antibody.
Fig. 2. A typical Class 3 cell has many stress fibres and low velocity. The arrow indicates the direction of translocation. Stress fibres are thick and parallel or oblique to the axis of the cell. The stain was a fluorescein conjugated anti-actin antibody.
Morphology of motile fibroblasts in vitro

Previous studies of fibroblast translocation have often focused on the movements of flattened cells that are triangular in outline (Abercrombie, 1980). We observed such 'classical' or tailed fibroblasts (TF) to measure usually between 160–200 μm in length and up to 100 μm across their base or leading edge (Figs. 2 and 6). The tapering tail was between 1/3 to 1/2 the length of the cell. Fan-shaped cells lacking a significant tail were also seen. But the TF was not the most common morphology we observed. In examining films of many hundreds of cells we more often saw a tailless form which resembled a half-moon or half-ellipse (HMF), (Fig. 7). The nucleus of the HMF lies posteriorly in a thickened zone which borders the trailing edge of the half-moon. The outline of a typical HMF occupied somewhat less surface area than that of a typical TF. Stationary cells were usually bipolar or broadly spread polygons. Some morphologies were intermediate between the distinct types described above.

Origin and duration of TF and HMF morphologies

A TF usually developed from a cell of poorly defined morphology that extended and attached a podial process to the substrate (16 of 24 cells studied, Fig. 6, A–D). In these 16 cells the tail arose from a lamellipodial process while the main mass moved slowly away from it (12 of the 16 cells moved more slowly than 0.5 μm/min). The process thus became a tail (Fig. 6). The process of tail formation took approximately 13 h. Tail development time occupied about one-third of the total time required for the full cycle of movements which culminated in tail snapping. In 3 of 24 cases, the tail formed from the leading edge of motile HMFs. Tails were seen to form in stationary cells of undefined morphology when one region of the cell adhered to the substrate while the main cell mass moved in the opposite direction (3 of 24 cases). In two cases, the main cell mass of a cell with the morphology of a TF reversed direction so that a region of the old leading edge was transformed into the tip.
Figs. 4, 5. Two cells in the same colony are stained with a fluorescein conjugated antibody to myosin. The cell in Fig. 4 has no stress fibres and a high velocity, while the cell in Fig. 5 has many stress fibres and a much lower velocity.
Fig. 6. In frames from a time lapse sequence of a fibroblast filmed during tail formation (A–D), cell stretching (E–I), and tail snapping (C–I), the dot is fixed point on the substrate. The open arrow indicates the posterior region of the cell. The solid arrow in C points to the leading edge and the solid arrow in I points to a tail-like region of the cell that was previously part of the leading edge (note C–F). Time in hours and minutes after the start of the sequence is shown at the lower right of each micrograph. Optics were Hoffman Modulation Contrast.
of the tail. Trajectories of HMFs and TFS studied to compare velocities were selected with the restriction that cells made no contacts during excursions.

The decision to form a tail is related to whether or not the substrate that underlies a cell has been previously crawled on. In two films of colonies developing from founders of the 10th and 30th PDLs, tail formation was studied during the first three days of filming. Twenty-one cells moved on previously used substrate while 20 did not. Of the 21 that did, twelve tails formed. Of the 20 that travelled on virgin substrate none developed tails. In two cases when a tail formed in a cell that lay partly on unused substrate and partly on previously travelled substrate, the tail formed in the region of the cell that was on used substrate.

TF and HMF shapes are interconvertible morphologies. An HMF can arise from cells of various morphologies that reorganize into the half-moon shape. After the tail of a TF snapped, the cell often transformed into an HMF. The persistence of each of these morphologies was studied in 34 cells in four films over the lifetime of each cell (Fig. 8). The HMF morphology prevailed for 29 ± 9% of the lifetimes of cells studied, while the TF morphology was adopted by cells for only 7 ± 6% of their lifetimes. These numbers account for only 36% of the lifetimes of the cells under study. Ground rules exclude those periods during which cells were in contact with other cells, and those periods during which cell morphologies were not assignable to either half-moon or tailed categories. While 24 of the cells spent at least part of their lives as both TFs and HMFs, 12 of the cells assumed the HMF shape at times but were never seen to assume the TF shape. On the other hand, cells that spent part of their lives as TFs always spent part as HMFs. Hence, the more usual morphology for the 1519 fibroblasts in this study was that of the HMF rather than the TF.

**Translocation of tailed and half-moon fibroblasts in vitro**

The translocation of a TF began just after the tail formed and the main cell mass moved in a direction opposite to that of the tip of the tail (Fig. 6 D–E). It consisted of 2 phases: phase 1 was the period during which the main cell mass executed a trajectory while the tail remained adherent to the substrate (Fig. 6 D–E); because the tip of the tail was fixed in position, the tail could be greatly stretched during this phase. During phase 2, the tail snapped forward (Fig. 6 E–I) and a portion of it sometimes broke off from the main cell mass. In analysing the phenomenon of TF translocation, we considered only those cells which were not in contact with other cells during translocation. The average duration and velocity of each phase of TF translocation is given in Table 1. Note that while the velocity of cell translocation during phase 2 (tail snapping or breaking) is about twice that of phase 1 (cell stretching), the velocity during phase 2 does not significantly increase the average velocity since the duration of phase 1 is 36 times longer than that of phase 2. After a single TF translocation, the fibroblast can usually assume a shape other than a TF and, of 33 TF translocation studied, 15 occurred only once during the lifetime of the cell. No cell was seen to translocate as a TF more than three times in its lifetime. Consecutive translocations as a TF were rare, although a few cells were observed to pass uninterrupted through several cycles of extension and tail snapping typical of the TF.
Fig. 7. In frames from a time lapse sequence (A–F) three half-moon fibroblasts (arrows in A and F) were filmed. The dot is a stationary point on the substrate. A stationary tailed fibroblast is seen to the left of cell number 1. Time in hours and minutes after the start of the sequence is shown at the right of each micrograph. The optics were Hoffman Modulation Contrast.
Locomotion of a HMF is not punctuated by a jerky movement like the tail snap that characterizes a TF translocation. The average HMF translocation lasted for 9.6 ± 1.3 h (n = 72), during which time the cell moved with an average velocity of 0.7 ± 0.1 μm/min, while the average velocity of a TF was 0.6 ± 0.1 μm/min.

![Graph](image)

**Fig. 8.** The percentage of lifetimes of 34 cells spent in half-moon (open bar) or tailed (shaded bar) morphologies. Cells, both in and out of cycle, spend more time in a half-moon than a tailed mode. Note that 12 cells never assume a tailed morphology.

**Table 1. Motility of tailed fibroblasts**

<table>
<thead>
<tr>
<th>Event</th>
<th>Duration (h)</th>
<th>Average velocity (μm/min)</th>
</tr>
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<tbody>
<tr>
<td>Phase I cell stretching</td>
<td>3.0 ± 1.2</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Phase II tail snapping or breaking</td>
<td>0.8 ± 0.4</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>TF translocation = phase I + phase II</td>
<td>3.9 ± 0.7</td>
<td>0.6 ± 0.1</td>
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</tbody>
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* All values are equal to the mean velocity ± 2 standard errors of the mean.

**The relationship between velocity and cycling state:**

In four films we identified 36 in-cycle cells and 23 out of cycle cells. We considered any cell that did not divide within 109 h out of cycle, (the average duration of a cell's life plus 3 standard errors of the mean was 109 h). For each in-cycle cell, we had a complete record of its velocity from its birth to the time it divided.

The average velocities of in and out of cycle cells were both 0.5 ± 0.1 μm/min (n = 36 for in-cycle cells and n = 23 for out of cycle cells). Both in and out of cycle cells adopted TF and HMF morphologies and as described above (Fig. 8) both spent more time moving in the HMF mode. There was also no significant difference in the average velocity of cells of different cycling states, nor was there a significant difference between cells moving as TFs or HMFs (Table 2).
**Table 2. Velocity of cell translocation as a function of cycling state and morphology**

<table>
<thead>
<tr>
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<th>Half-moon translocation</th>
<th>Tail translocation</th>
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<tbody>
<tr>
<td>In cycle</td>
<td>0.6 ± 0.1 (n = 26)</td>
<td>0.6 ± 0.1 (n = 14)</td>
</tr>
<tr>
<td>Out of cycle</td>
<td>0.8 ± 0.1 (n = 41)</td>
<td>0.6 ± 0.1 (n = 16)</td>
</tr>
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</table>

* All values are equal to the mean velocity ± 2 standard errors of the mean. All units are in μm/min.

**DISCUSSION**

Guesses about the nature of the engine responsible for the translocation of fibroblasts have been made mainly on the basis of structures visible in the light microscope and their chemical composition. Stress fibres have ranked high as potential components of an engine for cell translocation (for review, see Abercrombie, 1980). The fibroblast, however exhibits contractility somewhat like a muscle cell when it is not translocating (Gabbiani, 1979). Consequently, the presence alone of contractile structures in translocating cells does not prove that they serve as organelles of locomotion. Contractile functions are displayed in the course of wound healing (Gabbiani, 1979), in collagen remodelling, and in the contraction of artificial three-dimensional lattices (Bell *et al.* 1979a). In these activities fibroblasts extend and withdraw podial processes while the main mass of the cell remains fixed. The modelling and remodelling of connective tissues may depend mainly on the contraction of podia which interact with a pliable matrix of collagen or elastin fibrils which yield to the pull exerted by a cell process attached to it. On glass or plastic, the substrate does not yield; rather, since the cell process is attached firmly to an unyielding substrate when a podium is withdrawn, the attachments are broken and the cell process snaps and sometimes breaks. Actually, the mechanism of the retraction of a cell process occurring during translocation or during remodelling of a matrix may be similar and may involve a contractile event mediated by stress fibres, but it is necessary to assess the contribution of the event of translocation. Glycerinated models of fibroblasts to which ATP and Ca²⁺ are added (Hoffman-Berling, 1954, 1959) give evidence that both podial and tail processes undergo contraction when triggered. With polarization optics, we have observed stress fibre shortening in such models (Marek *et al.* unpublished results). However, as we have shown in the present paper, the contribution of tail snapping or shortening to translocation occurs over 22·0% of the translocation cycle and changes the average velocity only slightly. In fact, tail shortening and the tail itself are not necessary conditions for the translocation of 1519 fibroblasts even though they can contribute to it. This emerges from our study of the morphologies of translocating cells. The most frequently seen morphology is that of the HMF which has no tail, yet moves slightly faster than the TF. How then does a cell without a tail and without stress fibres posterior to the nucleus move? Even though we show an inverse relationship between the density of stress fibres and cell velocity, some are generally present. In HMFs, however, when stress fibres are seen, they are usually...
oriented perpendicular to the direction of cell movement. While we do not rule out the participation of stress fibres anterior to the nucleus in the translocation of TF or HMFs, we propose that they may not be needed. This suggestion is supported by the results above and by observations in living cells filmed with polarization optics. In many motile cells few, if any, stress fibres are present while stationary cells are heavily endowed with them (Bell et al. unpublished results). The crucial point is that while stress fibres probably play an essential role in contracting cell processes, they may not constitute a necessary condition for cell translocation.

The breadth of the leading edge of the HMF fibroblasts is similar to that of the highly motile fan-shaped cell observed by Badly et al. (1980) and Couchman & Rees (1979) who distinguished between two stages in the outgrowth of heart fibroblasts from tissue fragments. The first is one of extensive cell translocation and the second of cell division with little movement. These authors reported the absence of focal contacts in such highly motile cells, which suggests that less frictional resistance is experienced by the cells which lack focal contacts as they translocate over a substrate. Without focal contacts into which they are usually seen to insert (Weihing, 1979) it is difficult to see how stress fibres can work to pull the cell mass forward. If stress fibres are not an essential requirement for translocation, we raise the question of whether podial protrusion or, more properly, the factors which cause it are mainly accountable for translocation. In discussing the problem of the protrusive activity of fibroblasts, Abercrombie (1980) implicated cytoplasmic streaming and commented, 'there is no escaping the necessity of postulating such a flow'.

Recently Bell et al. (1980) presented evidence for cytoplasmic streaming associated with protrusive activity of fibroblasts. They filmed the intracellular translocation of polystyrene latex beads incorporated by cells and of mitochondria which move in axes perpendicular to the leading edge of moving cells and are sometimes swept around the leading edge in a circuit which takes them back toward the nucleus. They postulate the existence of a ubiquitous pump responsible for generating hydrodynamic flow like that present in the amoeba. Support for this idea that the engine or pump is widely distributed in the cell is provided by experiments which show that small enucleate fragments which form from cell tails or leading edges can translocate independently of the cell (Lewis et al. 1980). The nature of the pump, whether something like the microtrabecular lattice of Wolosewick & Porter (1979) or the meshwork of actin described by Heuser & Kirchhner (1980) and Temmink & Spieie (1980) or the actin meshwork associated with tubulin described by Schliwa & Van Blerkon (1981) in detergent extracted cells remains to be explained.

Two important conclusions which confirm previous findings can be drawn from our results on the morphology and velocity characteristics of cells of widely different PDLs. We find in cells of both low and high PDL similar intracclonal variations in cytostructure, velocity and cell morphology. Secondly, the cycling state of a cell, regardless of PDL, has little to do with its morphology; i.e. whether TF or HMF when it is translocating. Cells both in and out of cycle adopt the HMF morphology more frequently than the TF morphology. The average velocity of cells in these respective morphologies is also similar.
Fibroblast translocations

The role of the substrate as a determinant of cell morphology and behaviour is illustrated by the data relating tail formation to prior substrate use. We show that conditioning of the substrate by cells traversing it is a consistent requirement for tail formation. Tail formation may depend then on a specialized bond with the substrate which only prior cell use makes possible.

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