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Effects of LETS Glycoprotein on Cell Motility

Iqbal Unnisa Ali and Richard O. Hynes
Department of Biology and
Center for Cancer Research
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Summary

Addition of LETS glycoprotein to normal or transformed cells produces increased migration of the cells, as determined by formation of phagokinetic tracks on gold particle-coated coverslips. These tracks arise by a combination of phagocytosis of the gold particles and cellular migration. Increased motility is also evident on plastic in the absence of gold particles. The added LETS protein attaches to the cells in a fibrillar network, and binding is greater to normal than to transformed cells. The effects of LETS protein on migration are consistent with its effects on cell adhesion, morphology and cytoskeleton, and have potential implications for the determination of cellular migration in vivo.

Introduction

The molecular mechanism of locomotion of cells in vitro is complex and still poorly understood. Specialized surface microextensions are believed to be involved in probing the vicinity of the cell and determining the movement of the cell in a certain direction (Albrecht-Buehler, 1976; Albrecht-Buehler and Lancaster, 1976). The locomotion of cells is also greatly influenced by cell-cell and cell-substratum adhesive properties (Carter, 1965; Harris, 1973a; Martz and Steinberg, 1974), and by cellular asymmetry and components of the cytoskeleton (Goldman et al., 1973; Vasiliev and Gelfand, 1976; Albrecht-Buehler, 1977b). It seems probable that the surface membrane of the cells and its associated molecules are of importance in these phenomena. Recently, LETS (large, external, transformation-sensitive) glycoprotein, which is a major surface component of normal cells (Hynes, 1976), has been shown to confer upon transformed cells increased adhesivity, flattened and asymmetric morphology, and a more organized cytoskeleton (Yamada, Yamada and Pastan, 1976b; Ali et al., 1977). All these properties are very likely to affect locomotion of cells. It has been reported that LETS protein increased motility of a nonadhesive mutant cell line (Pouyssegur, Willingham and Pastan, 1977). LETS protein is also referred to as CSP (Yamada et al., 1976b) or fibronectin (Vaheri and Mosher, 1978).

The locomotory behavior of cells has been studied on glass substrata uniformly coated with colloidal gold particles. During attachment, spreading and movement, cells remove gold particles (some of which are ingested) from the substrate. The particle-free tracks left by moving cells have been named "phagokinetic tracks," since they are generated by a combination of phagocytosis and locomotion (Albrecht-Buehler, 1977a). We have observed the phagokinetic tracks of several normal and transformed cell lines before and after the addition of LETS protein. Our results indicate that the motility of cells is markedly increased after the addition of LETS protein.

Results

Phagokinetic tracks generated by several normal and transformed cell lines in the course of 2 days in the absence or presence of 50 μg/ml LETS protein are shown in Figure 1. Each cell line had a characteristic pattern of movement, as previously reported by Albrecht-Buehler (1977a). Under the conditions used, NIL8-HSV cells did not move at all and grew in the form of tightly packed colonies surrounded by particle-free rings cleared by the cells (Figure 1a). Upon addition of LETS protein, however, they showed a large increase in motility and cleared very wide areas free of gold particles (Figure 1b). NIL8 cells, which showed little movement in controls (Figure 1e), displayed enhanced motility after the addition of LETS protein resulting in the formation of extensive broad tracks (Figure 1f).

Figure 1g shows long straight tracks of 3T3 cells; their polyoma and SV40-transformed derivatives formed branched tracks (Figures 1i and 1k), as reported earlier (Albrecht-Buehler, 1977a). Upon addition of LETS protein to 3T3 and Py-3T3 cells, a general increase in the extent of track formation was observed (Figures 1h and 1l). SV-3T3 cells, however, showed a greater increase in locomotion than 3T3 or Py-3T3 cells, making large dark patches free of particles upon addition of LETS protein (Figure 1j). A rat cell line transformed with temperature-sensitive avian sarcoma virus made short and curved tracks at both nonpermissive (39°C) and permissive (34°C) temperatures (Figures 1m and 1o). After addition of LETS protein, the tracks formed at both temperatures were long, thin and straight (Figures 1n and 1p).

An increased accumulation of gold particles was observed inside and/or on the dorsal surface of LETS protein-treated cells as compared with untreated controls. This may be a result of larger track formation and increased clearing of gold particles which are then internalized or accumulated on the cell surface to a greater extent. In the

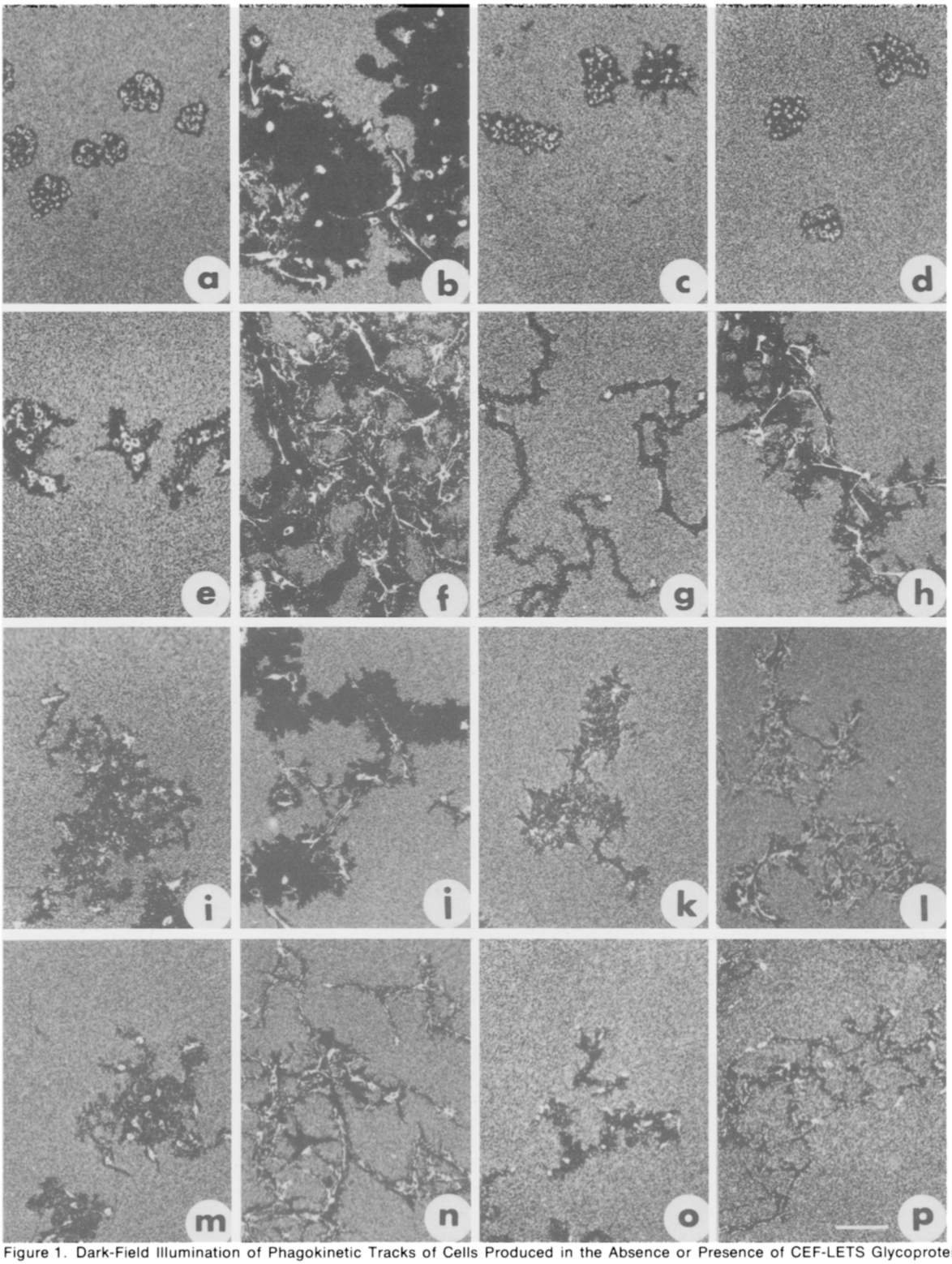

Figure 1. Dark-Field Illumination of Phagokinetic Tracks of Cells Produced in the Absence or Presence of CEF-LETS Glycoprotein on Gold-Coated Glass Coverslips

Cells were grown for 2 days without or with 50 μg/ml LETS protein (except c and d) which was added at the time of plating. Bar = 100 μm. (a) NIL8-HSV; (b) NIL8-HSV + CEF LETS protein; (c) NIL-HSV + 100 μg/ml LETS protein from NIL8-conditioned medium preabsorbed with gelatin-coupled Sepharose beads; (d) NIL-HSV + 100 μg/ml of thyroglobulin; (e) NIL8; (f) NIL8 + LETS protein; (g) 3T3; (h) 3T3 + LETS protein; (i) SV-3T3; (j) SV-3T3 + LETS protein; (k) Py-3T3; (l) Py-3T3 + LETS protein; (m) ts-rat at 39°C; (n) ts-rat at 39°C + LETS protein; (o) ts-rat at 34°C; (p) ts-rat at 34°C + LETS protein.

The cells appear as white dots because of their associated gold particles. White streaks visible in some panels are deposits of gold particles.

case of normal cells, many gold particles accumulated in the form of streaks and clusters along the tracks and in the vicinity of cells (Figures 1f, 1h and 1n). In contrast, tracks of transformed cells contained fewer such deposits in spite of clearing of large areas by these cells after the addition of LETS protein (Figures 1b, 1j and 1p). It is possible that large amounts of gold particles which are cleared from the tracks of transformed cells are either internalized or pinched off into the medium.

Similar effects on cell motility were obtained with LETS protein extracted from chick embryo cells or purified from NIL8-conditioned medium. The specificity of the effects was shown by the following experiments.

—When LETS protein preparations were absorbed with gelatin-Sepharose which binds LETS protein specifically (Engvall and Ruoslahti, 1977), the unabsorbed material was completely inactive (Figure 1c). LETS protein eluted from gelatin-Sepharose with 4 M urea was active (not shown).

—The effects of LETS protein were also abolished by pretreatment with trypsin (10 μg/ml for 10 min at 37°C) or with antiserum to LETS protein (not shown).

—Another high molecular weight glycoprotein, thyroglobulin at 100 μg/ml, had no effect on motility (Figure 1d).

When the formation of track patterns of NIL8-HSV cells was followed at different times after the addition of LETS protein, an increase in the size of particle-free rings was observed as early as 2 hr after the addition of LETS protein (data not shown). In 6 hr, cells cleared significantly larger areas than untreated controls (Figures 2a and 2b). By 12 and 24 hr, cells had made extensive tracks in the presence of LETS protein as opposed to control NIL8-HSV cells which made only particle-free rings around them (Figures 2c–2f).

The increased locomotion of LETS-treated cells was also found to be dependent upon the concentration of LETS protein added (Figure 2). Even at 5 μg/ml, LETS protein brought about an increase in the movement of NIL8-HSV cells (Figure 2g). At higher concentrations, cells cleared larger areas free of gold particles making longer and wider

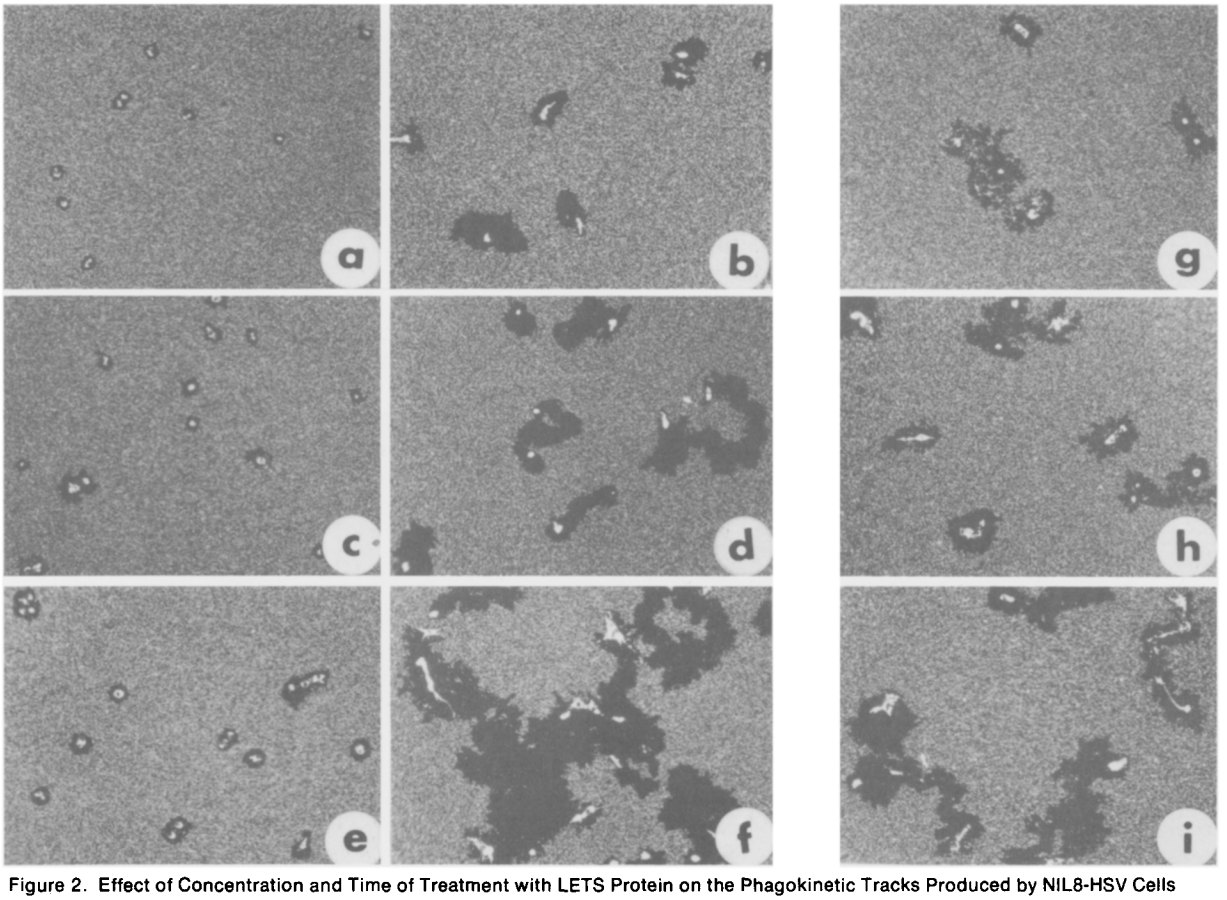

Figure 2. Effect of Concentration and Time of Treatment with LETS Protein on the Phagokinetic Tracks Produced by NIL8-HSV Cells

Magnification same as in Figure 1.

(a) Control and (b) + 50 μg/ml LETS protein; fixed 6 hr after plating. (c) Control and (d) + 50 μg/ml LETS protein; fixed 12 hr after plating. (e) Control and (f) + 50 μg/ml LETS protein; fixed 24 hr after plating. (g–i) + 5, 10 and 25 μg/ml LETS protein, respectively; fixed 24 hr after plating.

tracks (Figures 2h and 2i), and the effect was saturated at 50 μg/ml LETS protein. Increasing the LETS protein concentration to 75–100 μg/ml was without further effect (data not shown). Increased movement was also observed when NIL8-HSV cells were plated on coverslips which were preincubated for 24 hr in medium containing 50 μg/ml LETS protein, washed and transferred to medium without LETS protein (not shown).

The results observed in Figures 1 and 2 could be a reflection of increased migration or of increased phagocytosis of gold particles by the treated cells, or both. To distinguish between these possibilities, NIL8-HSV cells were plated in plastic dishes in the presence or absence of LETS protein (Figure 3). Untreated NIL8-HSV cells were nonmotile and grew in small colonies. Upon treatment with LETS protein, they acquired an asymmetric shape and were well separated from each other. This indicates that there is a direct effect of LETS protein on migration independent of any effects on phagocytosis of gold particles.

Figure 4 shows immunofluorescent staining of cells on gold particle-coated coverslips before and after the addition of LETS protein. Control NIL8-HSV cells showed no LETS fibrils (Figure 4b), and sparse growing NIL cells showed a small amount of staining in the form of fibers (Figure 4a), as reported previously (Mautner and Hynes, 1977). Fine fibrils staining for LETS protein were present beneath cells, between adjacent cells and around the edges of cell boundaries. In the presence of added LETS protein, a uniform coating of particles of LETS protein was present on the substratum.

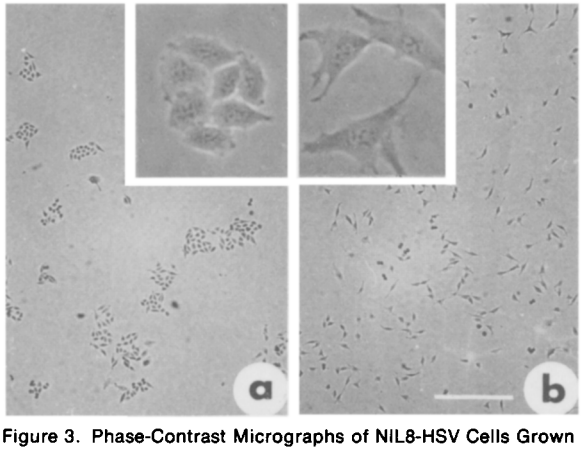

Figure 3. Phase-Contrast Micrographs of NIL8-HSV Cells Grown on Plastic in the Presence or Absence of LETS Glycoprotein

Pictures were taken 2 days after plating the cells. Bar = 250 μm. (a) Control culture with tightly packed colonies of nonmigrating cells; (b) + 50 μg/ml LETS protein; moving cells uniformly distributed on the substratum. Inserts show round morphology of control and asymmetric shape of treated cells at higher magnification.

These particles did not coincide with the gold particles. Both normal and transformed cells had LETS protein fibers on their surfaces after addition of this protein. The binding to normal cells, however, was much greater than binding to transformed cells (compare Figures 4c and 4f with Figures 4d and 4g). Quantitative measurements have confirmed the greater binding of LETS protein by normal cells (Hynes et al., 1978). Elaborate fibrillar networks of LETS protein were observed on NIL8 cells (Figures 4c and 4e) and ts-rat cells at 39°C (Figure 4f), as well as 3T3 cells (data not shown). Fibrils of LETS protein surrounded the cells, extending to distances far beyond the cells and forming extensive bridges between neighboring cells (Figure 4c). Figure 4f shows two ts-rat cells at 39°C with LETS fibers covering the cells and lying along the tracks. The presence of LETS fibrils in a broad track left behind by a NIL8 cell is shown in Figure 4e. Note that cells also removed the LETS particles.

LETS fibrils were formed to a lesser extent on transformed cells (Figures 4d and 4g). The fibrils were present primarily around the cells and sometimes between close neighbors. It is also evident that tracks of transformed cells, both wide (NIL8-HSV cells, Figure 4d; SV-3T3 cells, data not shown) and narrow (ts-rat cells at 37°C, Figure 4g), had fewer LETS protein fibrils and speckles. This may simply reflect the smaller, amounts of LETS fibrils bound to transformed cells.

Figure 5 shows that the LETS fibrils left behind in the tracks of cells are clearly distinct from the cells detected by phase optics. The deposits of LETS protein are frequently associated with gold particles (see Figures 1 and 5). Immunofluorescent staining with antisera to actin and myosin (not shown), however, was negative, demonstrating the observation that these deposits were not cell fragments. They presumably represent aggregates of cell surface material plus gold particles which are sloughed off the cells.

Discussion

There is evidence that locomotion of cells may be influenced by several factors, such as polarity of the cells (Vasiliev et al., 1970; Vasiliev and Gelfand, 1976), organized formation of adhesion plaques (Abercrombie, Heaysman and Pegrum, 1971; Harris, 1973b; Bell, 1977), surface protrusions such as filopodia (Albrecht-Buehler, 1976), number and size of the lamellipodia and ruffles (Ponten, 1975), and elements of the cytoskeletal system (Goldman, Schloss and Starger, 1976; Albrecht-Buehler, 1977b). The formation of cell substrate attachment areas seems to be of prime importance in determin-

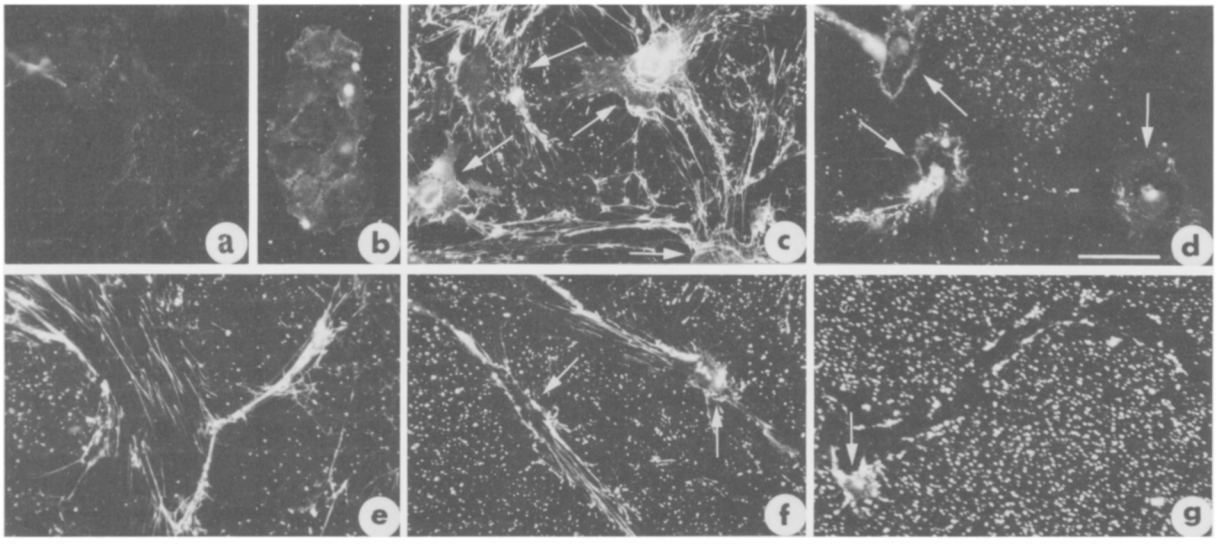

Figure 4. Fluorescent Micrographs of Cells and Their Phagokinetic Tracks Stained for LETS Protein

Cells were fixed and stained 2 days after growth in the presence of 50 μg/ml LETS protein (c–g). White spots in (c–g) represent particles of LETS protein adhering to coverslips in all LETS protein-treated cultures. Note that these particles did not coincide with gold particles. The arrows mark the positions of the cells. Bar = 50 μm.

(a) Control NIL cells; (b) control NIL-HSV cells; (c) NIL cells connected by extensive LETS fibrils; (d) broad tracks of NIL8-HSV cells with LETS fibrils around the cells; (e) LETS fibrils in an empty broad track left by a NIL8 cell; (f) narrow tracks of ts-rat cells at 39°C with LETS fibrils present on the cells and in the tracks; (g) narrow track of a ts-rat cell at 34°C. Note the relative absence of LETS fibrils and speckles from the tracks of transformed cells (d and g) and the presence of LETS fibrils in the tracks of normal cells (c, e, and f).

ing the shape and locomotory behavior of cells (Harris, 1973b; Bell, 1977). It has also recently been shown that the orientation of actin-containing bundles coincides with the direction of moving cells (Albrecht-Buehler, 1977b). Increased migration of cells observed in the presence of LETS protein may be through its effects on several of these parameters. An increase in adhesiveness has been shown to be one of the earliest effects upon addition of LETS glycoprotein (Ali et al., 1977). LETS protein also leads to a more organized cytoskeleton (Ali et al., 1977), especially the formation of well organized bundles of microfilaments (Ali et al., 1977; Willingham et al., 1977), and confers upon cells a more flattened and asymmetric morphology (Yamada et al., 1976b; Ali et al., 1977), the latter being a characteristic of migrating cells. In our experiments, cells which have a nonpolarized or rounded appearance on coverslips coated with gold particles (Figures 4a and 4b) show least movement (Figures 1a and 1e), whereas extremely asymmetric cells (for example, ts-rat plus LETS protein) show most translocation. It seems probable that the acquisition of asymmetric morphology of LETS-treated cells (Figure 3; Yamada et al.b, 1976; Ali et al., 1977) is a major contributor to the increased migration.

Yamada, Ohanian and Pastan (1976a) have reported a decrease in the numbers of ruffles and microvilli on transformed cells treated with LETS protein. The data of Yamada et al. (1976a), however, show a decrease in numbers of microvilli on the *upper* surface of cells; these are presumably not involved in cell locomotion. The reduction in marginal ruffles which they observed is probably consistent with our data. NIL-HSV cells have multiple ruffles all around the cell periphery (Figure 3, inset; Hynes, Destree and Mautner, 1976; Mautner and Hynes, 1977). After treatment with LETS protein, they become asymmetric, with ruffles confined to a few sections of their perimeters. One can then interpret the effects on locomotion in terms of cellular polarity. When ruffles are confined to limited region of the cell periphery, translocation occurs, whereas when they are distributed all around the cell, no net translocation occurs. This interpretation is also consistent with the effects of colchicine on cells (Gail and Boone, 1971; Goldman et al., 1973; Vasiliev and Gelfand, 1976). Colchicine prevents locomotion, but ruffling continues all around the edges of the cells.

The increased ability of cells to migrate in the presence of LETS glycoprotein seems to be independent of their capacity to phagocytose gold particles. This is shown by the fact that when LETS protein is added to NIL8-HSV cells, they migrate on plastic in the absence of gold particles (Figure 3). In addition, the formation of large arrays of LETS fibrils on the cells does not seem to be necessary for enhanced motility. Some transformed cells

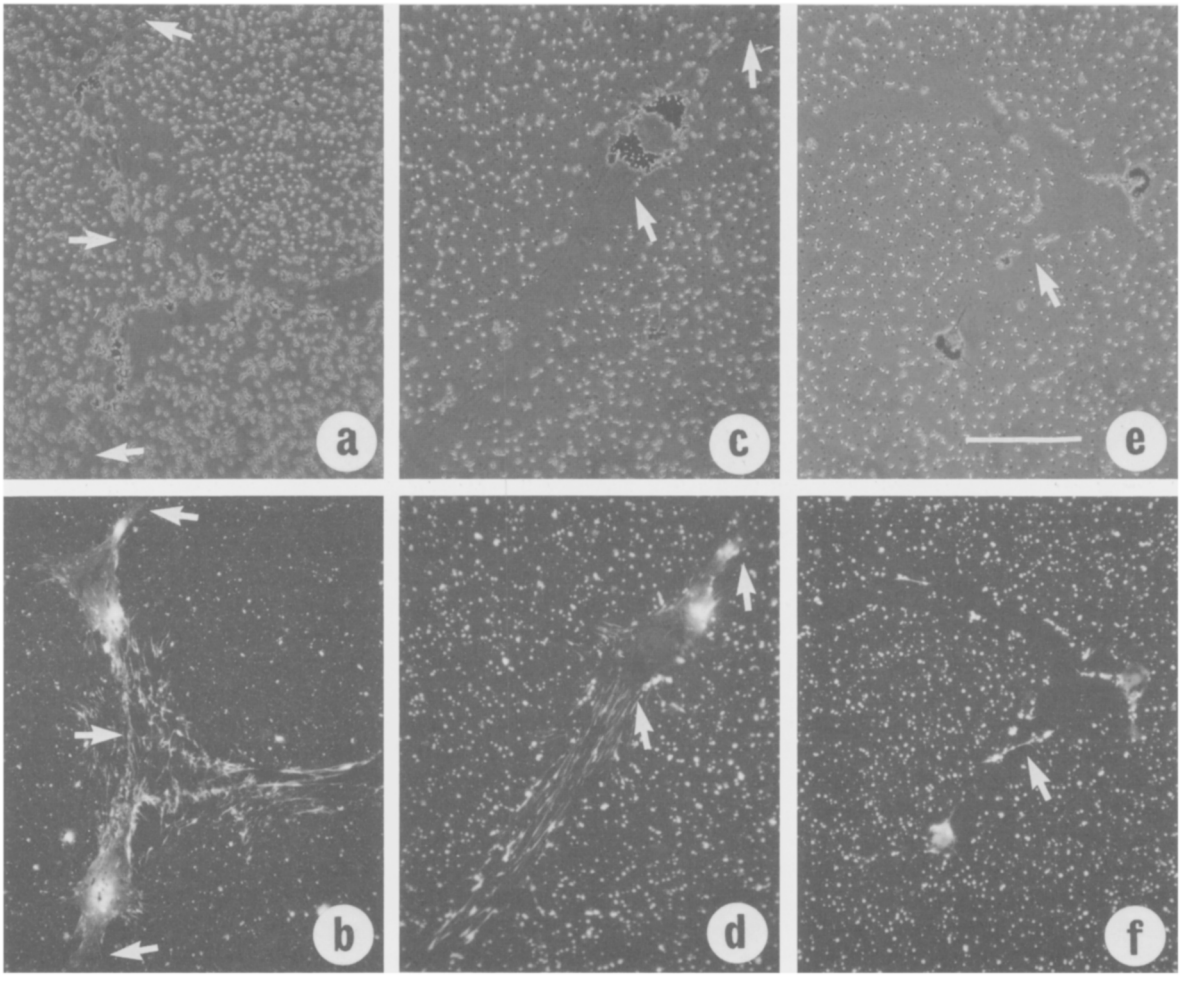

Figure 5. Deposition of LETS Protein in the Tracks of Cells

Phase-contrast (a, c and e) and immunofluorescence (b, d and f) analysis of cultures treated with 50 μg/ml CEF-LETS protein for 2 days. Bar = 50 μm.

(a and b) Pair of NIL8 cells separating after division. Note the LETS fibrils in the premitotic track and the coincident clumps of gold particles. The arrows mark the fronts of the cells and their connecting "tails."

(c and d) Single ts-rat cell cultured at 39°C. The circumnuclear accumulation of gold particles shows clearly. The track contains clusters of gold particles and LETS fibrils. The arrows mark the front and rear ends of the cell. The cell had presumably retracted its "tail" shortly before fixation.

(e and f) Pair of ts-rat cells separating after division. Cells were cultured at 34°C. Note the reduced amount of LETS protein left in the tracks as compared with normal cells (a–d). The arrows mark a deposit of LETS protein and gold particles.

which seem to migrate more and clear wider areas of gold particles than their normal counterparts have fewer LETS fibrils on their surface than do normal cells (compare Figures 4d with 4c). Immunofluorescent analysis of sparse cells on untreated coverslips shows that cells which migrate efficiently in the absence of added LETS protein do not form extensive arrays of LETS protein fibrils. LETS protein is predominantly found in areas of cell substratum contact and beneath the edges of the lamellae of spreading and migrating NIL8 cells (Mautner and Hynes, 1977; Hynes et al., 1978), and this has been confirmed for 3T3 and ts-rat cells (unpublished data). This seems, in all likelihood, to be the fraction which is necessary for migration. It is noteworthy that the increased migration can be induced by pretreatment of the substratum with LETS protein.

The presence of extensive fibrillar networks on confluent 3T3 cells was shown to interfere with the centripetal transport of gold particles on the dorsal surface of the cells (Albrecht-Buehler and Chen, 1977). Whether the same phenomenon occurs in cells treated with LETS protein over extended time periods remains to be seen. It has been postulated that centripetal flow has a role in migration (Harris, 1973c).

There are several possible implications of the

present results. LETS protein is widely distributed in connective tissues and basal lamellae, and it is conceivable that is has a role in determining migration of cells within embryos and within the body. It has been shown that cells respond to artificial gradients of adhesiveness (Carter, 1965; Harris, 1973a), and it has been suggested that controlled distributions of adhesion-promoting molecules could similarly influence migration in vivo (Gustafson and Wolpert, 1967; Trinkaus, 1976). LETS protein is a candidate for such a role.

Experimental Procedures

Cells used were hamster lines, NIL8 and a derivative, NIL8-HSV, transformed by hamster sarcoma virus (HSV) (Zavada and Macpherson, 1970; Ali et al., 1977), mouse lines, 3T3, SV3T3 and Py3T3 [a gift from Dr. Keith Burridge (Burridge, 1976)] and a rat line (Prasad, Zouzias and Basilico, 1976) transformed by the tsLA34 mutant of B77 (Toyoshima and Vogt, 1969). The last line was a gift from Dr. John Wyke (Imperial Cancer Research Fund, London, England).

Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% FCS. 500–1000 cells were plated in 35 mm plastic dishes containing 2 ml of growth medium and an 18 × 18 mm coverslip coated with gold particles. LETS protein was usually added at the time of plating the cells. The isolation and purification of LETS protein from chick embryo fibroblasts and from conditioned medium of NIL8 cells was as previously described (Yamada et al.b, 1976; Mautner and Hynes, 1977; Ali et al., 1977) and also by affinity chromatography on gelatin-Sepharose (Engvall and Ruoslahti, 1977). The purity of these preparations has been reported in the relevant publications. The preparation of colloidal gold particles and their binding to glass coverslips coated with bovine serum albumin (BSA) was according to the method described in detail by Albrecht-Buehler (1977a). Dark-field pictures were taken on a Zeiss microscope using a 6.3x objective lens. Immunofluorescent staining for LETS protein on gold particle-coated coverslips after formaldehyde fixation was as described elsewhere (Mautner and Hynes, 1977). Cells were not permeabilized with acetone for staining of LETS protein, but showed some tendency to become permeable without acetone treatment. Photographs were taken on a Zeiss microscope equipped with epifluorescence optics using a 40x objective lens and Plus-X film.

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