Microfilament-Organizing Centers in Areas of Cell Contact: Cytoskeletal Interactions during Cell Attachment and Locomotion

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ABSTRACT In this article we discuss three aspects of cell contact formation: (*a*) the molecular architecture of the cytomatrix in cell-to-substrate focal contacts, (*b*) the dynamic properties of membrane- and microfilament-associated proteins in the contact areas, and (*c*) the involvement of microtubules in the coordinated and directed formation of new substrate contacts during cell locomotion. We show that different microfilament-associated proteins exhibit distinct patterns of association with focal contacts: some proteins are specifically associated with focal contacts (vinculin and talin); α-actinin is enriched in the contact areas but also is present along the stress fibers and in the lamellipodium; actin and filamin are detected throughout the contact areas but in apparently reduced amounts compared with the associated stress fibers; and tropomyosin, myosin, and spectrin are either absent from the endofacial surfaces of contact areas or are present in only very small amounts. Fluorescence photobleaching recovery analyses performed with living cells microinjected with fluorescently labeled actin, vinculin, and α-actinin indicate that each of these proteins maintains a dynamic equilibrium between a soluble cytoplasmic pool and a membrane-bound fraction. Correlation of the distribution of vinculin and tubulin in motile fibroblasts to local movements of the leading edge of the same cells indicates that free-end microtubules extend into actively ruffling areas along the lamellipodium and that new vinculin-containing contacts are preferentially formed in these protruding regions.

Cells move by cycles of anterior membrane protrusion, establishment of contact with the underlying substrate, and retraction of the posterior trailing edge (for reviews, see references 1 and 2). The spatial and temporal coordination of these three major phases of the locomotory cycle is an essential element of directional cell motility.

Apparently, cells sense and identify external stimuli that affect their motility. These include chemotactic stimuli, contacts with neighboring cells, and changes in the texture or adhesivity of the substrate. In addition, a central mechanism probably exists that integrates these stimuli and coordinates the dynamic molecular events that occur in different domains of the cell. The most active region is the leading lamellipodium. This area is very rich in actin filaments in the form of dense webs and small bundles (3–7). Besides actin, the leading lamella contains α-actinin (8–10) and, often, filamin. The focal contacts with the substrate that are formed under the leading lamella are particularly rich in the cytoskeletal protein vinculin and eventually, as they mature, become associated with the termini of stress fibers (9, 11–16). In fact, we suggest that focal contacts and the associated vinculin serve as organizing centers for the assembly of actin-containing microfilament bundles (17–19). The trailing edge of the cell is a relatively stationary domain with large birefringent stress fibers and large focal contacts (20). From numerous observations made over the last two decades, we have learned that the fine interplay between protrusion, substrate attachment, and retraction is a dominant factor in cell locomotion.

In this article we present findings concerning the dynamic molecular interactions involved in the biogenesis of focal contacts and discuss mechanisms that may be responsible for directional motility. We consider the following questions:

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What are the cytoskeletal constituents of focal contacts? What are the molecular interrelationships and interactions between them? What are their dynamic rearrangements during contact formation? We also discuss why some areas along the lamellipodium move steadily forward while others protrude and then retract and the involvement of other cytoskeletal networks in setting the direction of the formation of new substrate contacts and, thus, of cell motility.

MATERIALS AND METHODS

To localize the various cytoskeletal proteins we for the most part have used indirect immunofluorescent labeling. Vinculin was localized with pure rabbit or guinea pig antibodies (9, 21) or with our own recently prepared monoclonal antibody, 11.5.5. Rabbit antibodies to chicken gizzard talin (22) and bovine brain spectrin (23) were kindly provided by K. Burridge, University of North Carolina, Chapel Hill. Antibodies to chick brain tubulin were raised in either rabbits or guinea pigs as described (24). Antibodies against myosin light chain were prepared in collaboration with Dr. J. DeMey, Janssen Pharmaceutica, Belgium. Antibodies to tropomyosin, filamin, and α-actinin, all from chicken gizzard smooth muscle, were raised in rabbits and affinity purified as described (9, 21, 25). Actin was localized either by indirect labeling using rabbit antibodies to chicken actin or by fluorescently labeled phalloidin, kindly supplied by Dr. H. Faulstich, Max Planck Institute, Heidelberg. Secondary antibodies all were raised in goats and coupled to rhodamine-lissamine sulfonyl chloride or to dichlorotriazinylaminofluorescein as described (24, 25). Fragmin from *Physarum* was isolated as described (26, 27).

Ventral membrane was prepared by the $ZnCl_2$ method (28), and fluorescence photobleaching recovery experiments on membrane components or cytoskeletal microinjected proteins were carried out as described (29–32).

Triple labeling, time-lapse cinematography, cell relocalization, and photographic multiple imaging are described elsewhere (33), and a detailed report is in preparation (Rinnerthaler, G., et al.).

RESULTS AND DISCUSSION

Cytoskeletal Components of Focal Contacts and Their Interactivity

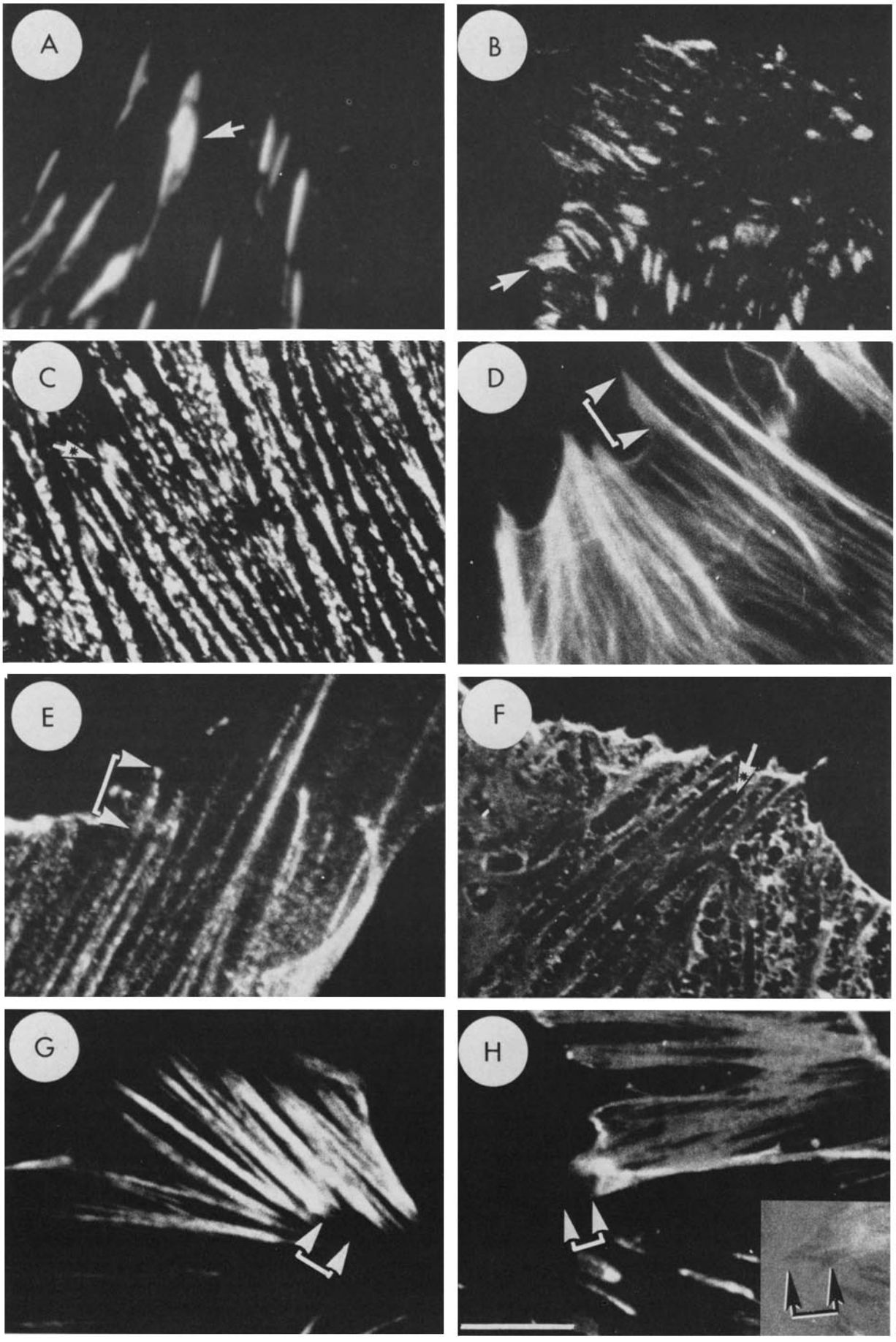
That focal contacts are associated with the termini of F-actin bundles has been demonstrated by both electron microscopy and immunocytochemical localization of actin, combined with interference reflection microscopy (11–16). Detailed immunocytochemical labeling has indicated that some microfilament-associated proteins are abundant near or at contact regions while others apparently are absent or present in low amounts. An example of such a comparison, using immunofluorescence for the localization of the actin and several of the associated proteins is presented in Fig 1: actin itself (*D*), as well as filamin (*E*), were detected in focal contacts, though in concentrations (as judged from the intensity of immunofluorescent labeling) that often were somewhat lower than those detected along the stress fibers; α-actinin (*C*) was abundant in or near focal contacts as well as in the unattached lamellepodium. Two proteins were specifically and predominantly associated with focal contacts (both mature and nascent), namely, vinculin (*A*) and the recently described 215,000 mol wt protein talin (*B*) (22). Spectrin (*F*), tropomyosin (*G*), and myosin (*H*) apparently were excluded from focal contact areas or were present there in very low concentrations.

Such light microscope studies provided valuable information on the general distribution of the different proteins, but, due to limited resolution, could not reveal the fine molecular interrelationships between them and their proximity to the plasma membrane. Several series of experiments have recently suggested that focal contacts contain two spatially and molecularly distinct domains: one is the microfilament-associated domain, which consists, at least, of F-actin, filamin, and α-actinin (provided that myosin, tropomyosin, and spectrin indeed are excluded); the other is the membrane-bound (actin-independent) domain, which contains vinculin and talin. The first indication that there is spatial segregation of the different cytoskeletal proteins was obtained several years ago using immunoelectron microscope localization of vinculin and other actin-binding proteins in focal contacts and related intercellular junctions (21, 34–36). We have shown, by single and double immunolabeling, for vinculin and α-actinin that, although both proteins are abundant near adherens junctions, vinculin is significantly and reproducibly closer to the plasma membrane (21, 35).

More recently, we have dissected the focal contact-bound filament bundle using fragminlike actin-severing proteins from *Physarum* or from pig smooth muscle (37). We have used this approach to determine whether the primary association of the various focal contact proteins was with actin filaments or with the endofacial surfaces of the membrane. A demonstration of such an experiment is presented in Fig. 2. Substrate-attached ventral membranes of cultured chicken gizzard fibroblasts were prepared as described (28), exposed to pure fragmin from either source, and double immunolabeled for actin and the various actin-associated proteins.

As demonstrated by the few examples shown in Fig. 2 (for additional information see reference 37), the treatment resulted in rapid and essentially complete disappearance of actin bundles from the membrane (compare the control sample, treated only with "fragmin buffer," without fragmin (Fig. 2, *B* and *F*) and the fragmin-treated sample (Fig. 2, *D* and *H*). Among the various actin-associated proteins tested, both vinculin and talin were fully retained on the actin-depleted membranes (Fig. 2, *C* and *G*, respectively), while α-actinin, filamin, and tropomyosin were highly sensitive (37). We therefore have concluded that the former two proteins are components of the membrane-bound "plaque" that serves as an anchor for the termini of actin bundles, while actin and the other associated proteins are peripherally associated with that plaque. The fine molecular interrelationships between vinculin and talin are still not clear, though some recent in vitro studies suggest that the two are involved in different

FIGURE 1 Immunofluorescent localization of different microfilament-associated proteins in relation to cell-substrate focal contacts. In all cases we prepared ventral membranes of cultured chick gizzard fibroblasts, labeled them for one microfilament-bound protein, and localized focal contacts on the same membranes using interference-reflection optics (for an example, see *inset* in *H*). The proteins localized were (*A*) vinculin, (*B*) talin, (*C*) α-actinin, (*D*) actin, (*E*) filamin, (*F*) spectrin, (*G*) tropomyosin, and (*H*) myosin. The single and twin arrows mark the location of focal contacts. Notice the specific localization of vinculin and talin in focal contacts and the enrichment of α-actinin. Actin and filamin are detected in, or near, contact regions, though in somewhat reduced amounts compared with stress fibers. Tropomyosin, spectrin, and myosin are essentially excluded. The *inset* in *H* is the interference-reflection image of the antimyosin-labeled cell in *H*. Bar, 5 μm.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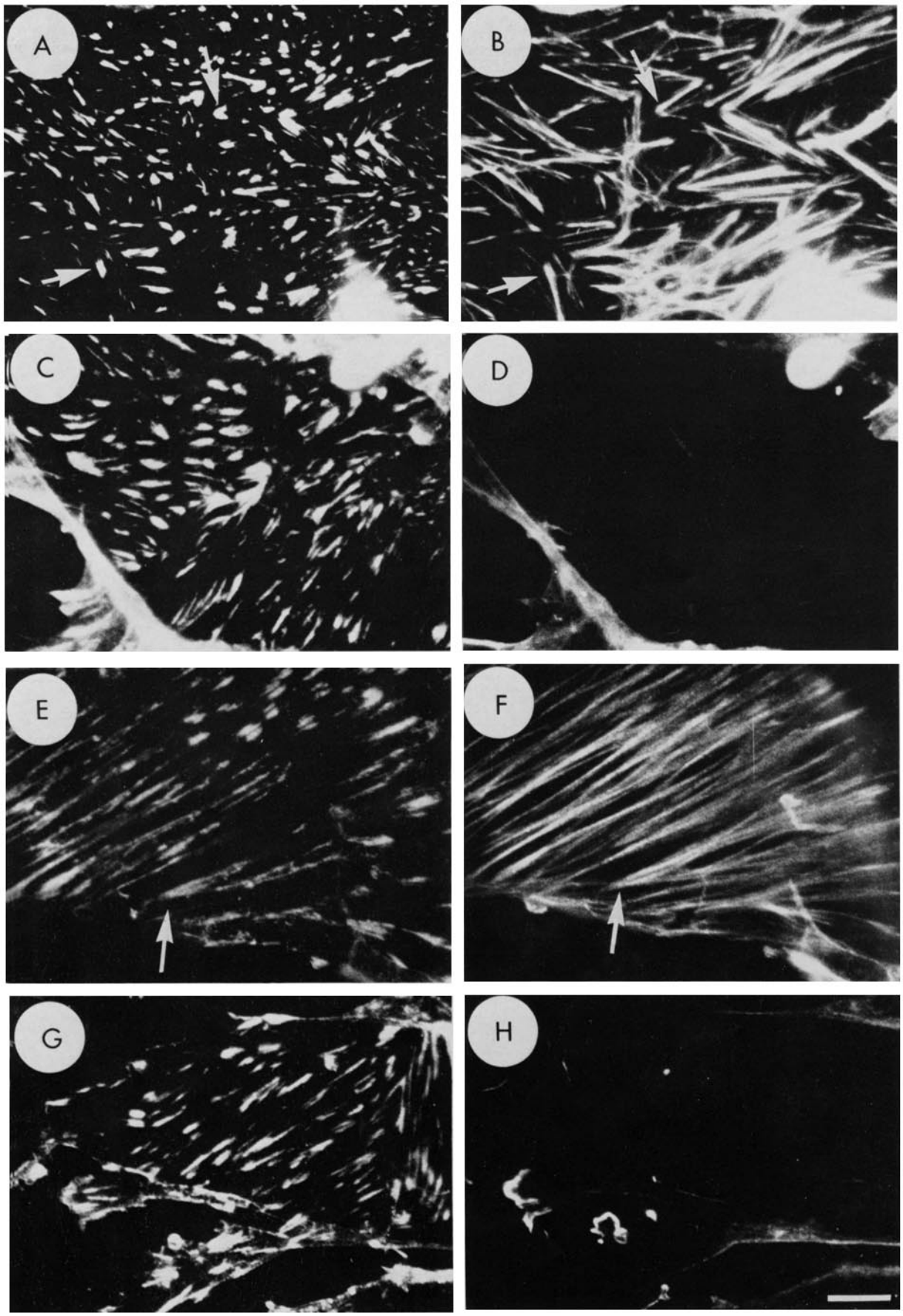
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molecular interactions. Vinculin was shown to bind to actin (38–40) as well as to additional proteins with apparent polypeptide molecular weights of about 130,000 and 220,000 (possibly vinculin and talin, respectively) (38). Studies by K. Burridge and P. Mangeat (personal communication) indicate that talin binds to vinculin. We have obtained some indirect evidence that vinculin also self-aggregates in focal contacts. We have found that rhodamine- or fluorescein-labeled vinculin may bind to and "decorate" focal contacts in opened-up ventral membrane preparations. Unexpectedly, we also found, however, that this binding was not easily inhibited by an excess of unlabeled vinculin (in contrast to the binding of rhodamine-labeled α-actinin, which was inhibited by the respective unlabeled protein) (10). A likely explanation of this observation is that vinculin self-aggregates locally on the membrane. This possibility, however, still requires experimental substantiation. Thus, there are several possible models for vinculin-talin interaction. Talin may be directly anchored to the membrane and bind vinculin through another site, and the latter may bind to and organize actin filaments. Alternatively, vinculin may be bound to the membrane independent of talin and even provide an anchor for both talin and actin. Additional experiments, including double immunoelectron microscopy and microinjection of antibodies to vinculin or talin into living cells, may help to resolve these questions.

In this discussion of focal contact components we have made no reference to the integral membrane components of the focal contact or of related zonula adherens-type intercellular junctions. Such components are undoubtedly extremely important elements in intercellular recognition and in the transmission of contact signals into cells. Unfortunately, in spite of intense efforts devoted to the isolation and characterization of junctional components, the integral membrane proteins of these junctions have not been identified.

Biogenesis of Adherens Junctions—Dynamic Aspects

The studies described above provided a rather static picture of the junction, its various cytoskeletal constituents, and the biochemical interrelationships between them. We would like to consider some dynamic aspects of junction formation, namely, the sequence of molecular events leading from the establishment of a new contact to the development of a mature, stress-fiber-bound focal contact.

Several years ago, we proposed that this process involves a cascade of nucleation reactions: the first step, according to that model, is the formation of small local associations between membrane protein(s) ("receptors") and the substrate. This interaction may lead to the aggregation of the membrane receptors and their immobilization in the small contact area. This clustering would induce the second set of events leading to the binding of soluble, cytoplasmic vinculin (and possibly talin) to the membrane. The vinculin-rich area on the membrane may subsequently function as a nucleation center for the assembly of actin bundles (for a more detailed discussion of the model, see references 17 and 18).

The temporal sequence of cytoskeletal reorganization during contact formation as revealed by fluorescence microscopy agrees with the proposed model. As described (17), the organization of vinculin on the membrane in newly formed contacts usually precedes the assembly of actin into bundles at the same sites. This also is true of the nascent substrate contacts formed under the protruding leading lamella of motile cells. In these areas, we often have observed small vinculin-containing streaks or small plaques under actively ruffling membranes (see below). In many instances, actin did not show any local accumulation in these areas or was present there in low concentrations.

More direct information regarding the dynamic rearrangements in adherens junctions was obtained in studies with cell models or living cells using the fluorescence photobleaching recovery approach. We have shown that the contact with the substrate does not lead to "freezing" or complete immobilization of membrane components (for details, see reference 32). Membrane lipids whose dynamic properties were measured using a lipid probe, WW591, moved freely through focal contacts in substrate-attached membranes, though at a somewhat reduced rate (the diffusion coefficient in unattached membranes was 12.8×10^{-9} cm^2/s, compared with 7.7×10^{-9} cm^2/s in focal contacts). The recovery of fluorescence, however, was complete, indicating that the lipid probe was completely mobile in the focal contact. The lateral diffusion of membrane proteins was analyzed with ventral membranes of chick fibroblasts exogenously labeled with rhodamine. The fluorescence photobleaching recovery data indicated that the lateral diffusion of the rhodamine-labeled membrane proteins in focal contacts was reduced in comparison to that measured in unattached areas (0.8×10^{-9} cm^2/s and 1.4×10^{-9} cm^2/s, respectively) (32). A major difference was found, however, between the extent of recovery of proteins in attached and free membrane regions. In the unattached regions nearly all the fluorescence rapidly recovered after local bleaching, whereas a significant proportion (~50%) of the labeled proteins in the contact areas did not recover. We thus concluded that two comparable populations of membrane proteins exist in focal contacts; one is free to move through that region, whereas the other is immobile, possibly due to the interaction with the solid substrate, with the cytoskeleton, or with both.

Similar dynamic behavior also has been found for the junction-associated cytoskeletal proteins actin, α-actinin, and vinculin. Fluorescence photobleaching recovery experiments with these components could not be performed with model membranes and therefore were carried out with living cells microinjected with these proteins labeled with either fluores-

FIGURE 2 Dissection between membrane-bound and microfilament-bound components of focal contacts. Ventral membranes were treated for 10 min with smooth-muscle fragmin from pig stomach, 10 μg/ml in fragmin buffer (37), (*C*, *D*, *G*, and *H*) or with "fragmin buffer" only (*A*, *B*, *E*, and *F*). The membranes were then fixed and double-labeled for vinculin (*A* and *C*) and actin (*B* and *D*) or for talin (*E* and *G*) and actin (*F* and *H*). The reagents used were rabbit antibodies for vinculin (9) and for talin (22) as first reagents and rhodamine-labeled goat antimouse Ig as the second. Actin was localized with fluorescein-conjugated phalloidin. Notice the disappearance of actin after fragmin treatments and the stability of both vinculin and talin to this treatment. Other microfilament-associated proteins (filamin, α-actinin, tropomyosin, and myosin) were highly sensitive to fragmin treatment. Arrows in the matched photographs point to the same locations. Bar, 10 μm.

TABLE I

Mobility of Rhodamine-labeled Actin, α-Actinin, and Vinculin in Microinjected Chicken Gizzard Fibroblasts as Measured by Fluorescence Photobleaching Recovery (19)

Protein*	Cellular domain	Diffusion coefficient	Fractional recovery	Half-time for fluorescence recovery in the "immobile" areas
		$D(cm^2/s) \times 10^{-9}$	%	
Actin	Focal contact or stress fibers	3.1 ± 1.1 (30)	18 ± 7	4.1 ± 2.8 (22)
α-Actinin	Focal contact	2.8 ± 1.0 (10)	40 ± 10	2.7 ± 1.2 (6)
Vinculin	Focal contact	3.5 ± 1.2 (20)	43 ± 8	2.1 ± 0.9 (16)
Actin	Interfibrillary	3.2 ± 1.2 (35)	65 ± 13	N.D.
α-Actinin	Interfibrillary	2.5 ± 1.4 (12)	76 ± 5	N.D.
Vinculin	Interfibrillary	2.9 ± 1.1 (30)	~80	N.D.

Number of determinations is indicated in parentheses. *N.D.*, not determined.

* Irrelevant proteins such as bovine serum albumin and goat immunoglobulin had diffusion coefficients of about 6×10^{-9} cm²/s and exhibited complete recovery regardless of the concentration or site of fluorescence photobleaching recovery measurement.

cein or rhodamine (references 19 and 31 and Kreis, T. E., J. Schlessinger, and B. Geiger, manuscript in preparation). Shortly after injection, each of the proteins became incorporated into the stress fibers and their focal contact-bound termini. Fluorescence photobleaching recovery analysis of these microinjected cells indicated that each protein was present in two dynamically distinct pools: a soluble, diffusible pool in the cytoplasm and another pool immobilized in focal contacts (for actin and α-actinin, also in stress fibers). Analysis of the fluorescence photobleaching recovery data indicated that the two pools maintain a dynamic equilibrium. Thus, when defined segments of focal contacts were photobleached, the fluorescence recovered slowly with a half-time of recovery of several minutes (see Table I).

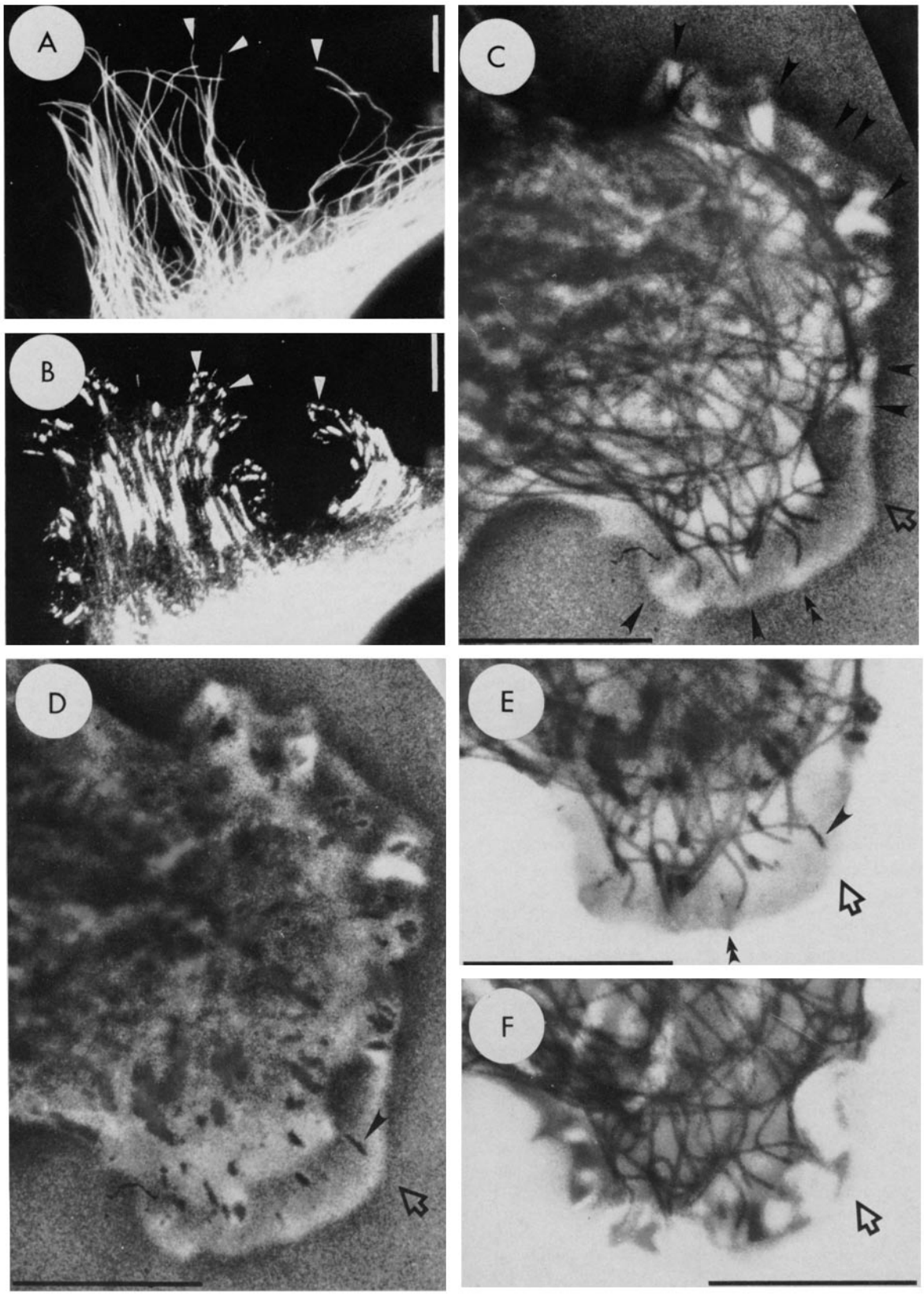
The findings described above concerning the spatial and temporal sequence of organization of focal contact components agree with the idea that junctional specialization is initiated by local contacts that induce vinculin and talin binding to the membrane and the formation of microfilament-organizing centers in those areas.

Involvement of Microtubules in the Formation of Stable Substrate Contacts at the Leading Edge of Motile Fibroblasts

It is generally accepted that microfilaments are intimately involved in the force-generating system necessary for cell locomotion. However, the factors involved in the polarization of cells, an essential step in directional locomotion, are largely unknown. Attempts to correlate the organization of cytomatrix components with the dynamic properties of the same cells were usually frustrated by two major obstacles: the apparent complexity and irregularity of the cytomatrix and the absence of direct analyses of the cytoskeletal organization and active lamellipodium movements in the same cell.

Time-lapse cinematography of cultured chick fibroblasts has revealed several types of movement of the lamellipodium, including continuous forward protrusion, protrusion followed by withdrawal, and ruffling activity (1, 2). To correlate the exact movements of the leading lamella before or at the time of fixation to the cytoarchitecture we have plated chick heart fibroblasts onto glass coverslips in a modified flow-through Dvorak-Stotler chamber and maintained the culture at 37°C on the stage of the microscope. After time-lapse cinematography, the cells were fixed in situ with a Triton-glutaraldehyde mixture (for details, see references 3 and 33). The proper fixation was of critical importance for the preservation of the delicate structures of ruffles and the lamellipodia as well as of the integrity of microtubules and other cytoskeletal structures. After fixation the cultures were double or triple labeled to visualize the relative distributions of different cytoskeletal proteins. For triple labeling, we first double labeled the cells using rabbit and either guinea pig or mouse antibodies for tubulin and vinculin, respectively. After photography, the fluorescein fluorescence was bleached and the cultures were further stained for actin using fluorescein-coupled phalloidin. With this approach we were able to localize within the same cells, actin, tubulin, and vinculin, reveal the location of sub-

FIGURE 3 Localization of tubulin, vinculin, and actin in the leading lamella of chick fibroblast. (*A* and *B*) Double immunofluorescent labeling of the same cell for tubulin (*A*) and vinculin (*B*). The arrowheads point to free-end microtubules that extend into the cell periphery with many vinculin-containing streaks. Arrowheads point to the same locations. (*C–F*) Multiple imaging of tubulin, vinculin, and actin by phase contrast and interference contrast microscopy. Details on the multiple imaging will be provided elsewhere (Rinnerthaler, G., et al., manuscript in preparation), and only the general principles are outlined here: cells were fixed and triple-labeled as described, and individual photographs taken on 35-mm black-and-white film. The negatives or positive transparencies prepared from them were then matched and used to print a combined picture. (*C*) Combined negative images of tubulin immunofluorescence and phase contrast. Note the free-end microtubules that extend into the lamellipodium in the lower part of the cell. The arrowheads point to phase-dense ruffling membranes (bright in the negative-image picture). (*D*) Combined negative image of vinculin immunofluorescence and phase contrast as above. The arrowhead matched with the arrowhead in *E* points to a vinculin-containing streak at the base of the leading lamella. (*E*) Combined negative-image photograph of tubulin, vinculin, and actin. The first two are the same as in *C* and *D*. Actin (diffuse gray) is enriched in the leading edge and photographed slightly above substrate level. Notice the small actin-containing ruffle (double arrowhead, matched with double arrowhead in *C*), which is colocalized with a small vinculin-containing streak and the free end of a microtubule. (*F*) Combined negative image of tubulin superimposed on positive image of interference reflection photomicrograph. Notice the fine radial close contacts under the leading edge. The hollow arrowheads in *C–F* points to the same area. Bars, 10 μm.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strate contacts (by interference reflection microscopy), and relate all these to the movements of the leading edge before or at the time of fixation. Experimental details on the cinematography, culture conditions, methods of rapid relocalization of cells, fixation, triple labeling, and photographic multiple imaging are given elsewhere (33 and Rinnerthaler, G., et al., manuscript in preparation).

Localization of microtubules indicated that they may be organized in two sharply distinct forms. Most microtubules extend from the perinuclear area toward the periphery and bend back or sidewise near the base of the lamellipodium. In some areas, however, individual microtubules apparently end near or within the leading lamella. Colocalization of tubulin and vinculin (Fig. 3) indicates that most of these free-end microtubule terminate very close to peripheral vinculin-containing sites. These vinculin-containing plaques at the leading edge seem to be the primordial forms of focal contacts. Examination of the time-lapse movies of many cells has indicated that both vinculin patches and the free ends of the microtubules at the periphery are closely related to local ruffling of the membrane before fixation. These interrelationships between ruffling, initiation of contact, organization of vinculin, and extension of microtubules into the lamellipodium are demonstrated by the set of photographs processed for multiple imaging in Fig. 3, *C–F* (for details, see legend to Fig. 3).

Frame-by-frame analysis of the phase-contrast images before fixation indicate that new vinculin-rich contacts are formed under or near radial membrane ruffles along the leading edge. These nascent vinculin-containing contacts were detected only about 1 min after initiation of ruffling. Microtubules also were detected in those areas close to the vinculin streaks, as pointed out above and as shown in Fig. 3. These microtubules, however, were detected earlier than vinculin, within 10–20 s of initiation of ruffling. Apparently, vinculin-containing contacts are not established only in ruffling areas, and we have seen many examples of new attachments formed under ruffle-free filopodial or lamellipodial extensions (not shown). The rate of vinculin organization in these sites is similar, and an interval of ~1 min was found between local protrusion and the apparent local association of vinculin with the membrane.

The findings presented here provide some insight into the involvement of microtubules in cell motility and the physiological significance of interactions between molecularly and structurally distinct components of the cytomatrix (in this case, tubulin and the vinculin-containing nascent contact). The exact role and mode of action of microtubules is not yet clear. The findings of others (41–46) and our own observations suggest that microtubules are not essential for establishment of substrate contacts or for the formation of membrane protrusions but are necessary for directional movements. This suggests that microtubules play a role in the spatial coordination of polarized contact formation. A simple mechanism that would correspond to our observations and to the model outlined above is that penetration of microtubules into certain areas of the lamellipodium or ruffles stabilizes these membrane extensions and prevents their retraction toward the cell body. Maintenance of these protrusions and prevention of their retraction for at least 1 min is necessary for the establishment of stable contacts leading to the induction of transmembrane association with vinculin and the initiation of actin bundle assembly. Obviously, other possibilities cannot be excluded, including a direct effect of free-end microtubules on vinculin and actin assembly and involvement of other elements of the cytomatrix.

We have discussed here three major aspects of cell anchorage and motility: the structure of cell-contact areas, their molecular dynamics, and their coordinated assembly during cell movement. Obviously, many of the molecular details necessary for a more complete understanding of the mechanisms involved in cell motility are yet to be explored. Among these are the nature of the contact receptors and the "transmembrane signal," the regulation of the dynamic equilibrium between the soluble and membrane-bound fractions of cytoskeletal proteins, the mechanism of peripheral microtubule extension, and the details of interfibrillary interactions within the cytomatrix.

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REFERENCES

1. Trinkaus, J. P. 1976. On the mechanism of metazoan cell movement. *In* The Cell Surface in Animal Embryogenesis and Development. G. Poste and G. L. Nicolson, editors. Elsevier/North Holland Biomedical Press, Amsterdam. 225–239.
2. Abercrombie, M. 1980. The crawling movement of metazoan cells. *Proc. R. Soc. Lond. B Biol. Sci.* 207:129–147.
3. Small, J. V. 1981. Organization of actin in the leading edge of cultured cells: influence of osmium tetroxide and dehydration on the ultrastructure of action meshworks. *J. Cell Biol.* 91:695–705.
4. Abercrombie, M., J. E. M. Heaysman, and S. M. Pegrum. 1970. The locomotion of fibroblasts in culture. I. Movements of the leading edge. *Exp. Cell Res.* 59:393–398.
5. Abercrombie, M., J. E. M. Heaysman, and S. M. Pegrum. 1971. The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. *Exp. Cell Res.* 67:359–367.
6. Lindberg, U., L. Carlsson, F. Markey, and L. E. Nystrom. 1979. The unpolymerized form of actin in non-muscle cells. *Methods Achiev. Exp. Pathol.* 8:143–170.
7. Small, J. V., G. Rinnerthaler, and H. Hinssen. 1982. Organization of actin meshworks in cultured cells: the leading edge. *Cold Spring Harbor Symp. Quant. Biol.* 46:599–611.
8. Lazarides, E., and K. Burridge. 1975. α-actinin: immunofluorescent localization of a muscle structural protein in nonmuscle cells. *Cell.* 6:289–298.
9. Geiger, B. 1979. A 130K protein from chicken gizzard: its localization at the termini of microfilament bundles in cultured chicken cells. *Cell.* 18:193–205.
10. Geiger, B. 1981. The association of rhodamine-labeled α-actinin with actin bundles in demembranated cells. *Cell Biol. Int. Rep.* 5:627–634.
11. Abercrombie, M., and G. A. Dunn. 1975. Adhesions of fibroblasts to substratum during contact inhibition observed by interference reflection microscopy. *Exp. Cell Res.* 92:57–62.
12. Izzard, C. S., and L. R. Lochner. 1976. Cell-to-substrate contacts in living fibroblasts. An interference reflexion study with an evaluation of the technique. *J. Cell Sci.* 27:129–159.
13. Heath, J. P., and G. A. Dunn. 1978. Cell to substratum contacts of chick fibroblasts and their relation to the microfilament system. A correlated interference reflection and high-voltage electron microscopic study. *J. Cell Sci.* 29:197–212.
14. Izzard, C. S., and L. R. Lochner. 1980. Formation of cell-to-substrate contacts during fibroblasts motility: an interference-reflection study. *J. Cell Sci.* 42:81–116.
15. Heaysman, J. E. M., and S. M. Pegrum. 1982. Early cell contacts in culture. *In* Cell Behaviour. R. Bellairs, A. Curtis, and G. Dunn, editors. Cambridge University Press, U.K. 49–76.
16. Gingell, D. 1981. The interpretation of interference-reflection images of spread cells: significant contribution from the peripheral cytoplasm. *J. Cell Sci.* 49:237–247.
17. Geiger, B. 1981. Transmembrane linkage and cell attachment: the role of vinculin. *In* International Cell Biology 1980–1981. H. G. Schweiger, editor. Springer-Verlag, Heidelberg.
18. Geiger, B. 1982. The involvement of vinculin in contact-induced cytoskeletal interactions. *Cold Spring Harbor Symp. Quant. Biol.* 46:671–682.
19. Geiger, B., Z. Avnur, T. E. Kreis, and J. Schlessinger. 1984. The dynamics of cytoskeletal organization in areas of cell contact. *In* Cell and Muscle Motility, Vol 5. J. W. Shay editor. Plenum Publishing Corp., New York. 195–235.
20. Chen, W. T. 1981. Mechanism of retraction of the trailing edge during fibroblast movement. *J. Cell Biol.* 90:187–200.
21. Geiger, B., A. H. Dutton, K. T. Tokuyasu, and S. J. Singer. 1981. Immunoelectron microscopic studies of membrane-microfilament interactions. The distributions of α-actinin, tropomyosin, and vinculin in intestinal epithelial brush border and in chicken gizzard smooth muscle. *J. Cell Biol.* 91:614–628.
22. Burridge, K., and L. Connell. 1983. A new protein of adhesion plaques and ruffling membranes. *J. Cell Biol.* 97:359–367.
23. Burridge, K., T. Kelly, and P. Mangeat. 1982. Nonerythrocyte spectrins: actin-membrane attachment proteins occurring in many cell types. *J. Cell Biol.* 95:478–486.
24. Geiger, B., and S. J. Singer. 1980. Association of microtubules and intermediate filaments in chicken gizzard cells as detected by double immunofluorescence. *Proc. Natl. Acad. Sci. USA.* 77:4769–4773.
25. Geiger, B., and S. J. Singer. 1979. The participation of α-actinin in the capping of cell-membrane components. *Cells.* 16:213–222.

26. Hinssen, H. 1981. An actin-modulating protein from *Physarum polycephalum*. I. Isolation and purification. *Eur. J. Cell Biol.* 23:225–233.
27. Hinssen, H. 1981. An actin-modulating protein from *Physarum polycephalum*. II. Ca^{+2}-dependence and other properties. *Eur. J. Cell Biol.* 23:234–240.
28. Avnur, Z., and B. Geiger. 1981. Substrate-attached membranes of cultured cells: isolation and characterization of ventral cell membranes and the associated cytoskeleton. *J. Mol. Biol.* 153:361–379.
29. Schlessinger, J., D. F. Koppel, D. Axelrod, K. Jacobson, W. W. Webb, and E. L. Elson. 1976. Lateral transport on cell membrane. The mobility of concanavalin A receptors on myoblasts. *Proc. Natl. Acad. Sci. USA.* 73:2409–2413.
30. Schlessinger, J., D. Axelrod, D. E. Koppel, W. W. Webb, and E. L. Elson. 1977. Lateral transport of a lipid probe and labeled proteins on a cell membrane. *Science (Wash. DC).* 195:307–309.
31. Kreis, T. E., B. Geiger, and J. Schlessinger. 1982. Mobility of microinjected rhodamine actin within living chicken gizzard cells determined by fluorescence photobleaching recovery. *Cell.* 29:835–845.
32. Geiger, B., Z. Avnur, and J. Schlessinger. 1982. Restricted mobility of membrane constituents in cell substrate focal contacts of chicken fibroblasts. *J. Cell Biol.* 93:495–500.
33. Small, J. V., G. Rinnerthaler, Z. Avnur, and B. Geiger. 1983. Cytoarchitectural changes associated with fibroblast lomocotion: involvement of ruffling and microtubules in the establishment of new substrate contacts at the leading edge. *In* Proceedings of the 1st International Congress on Contractile Proteins. Sarsari, editor. Temple University. In press.
34. Geiger, B., K. T. Tokuyasu, A. H. Dutton, and S. J. Singer. 1980. Vinculin, an intracellular protein localized at specialized sites where microfilament bundles terminate at cell membranes. *Proc. Natl. Acad. Sci. USA.* 77:4127–4131.
35. Tokuyasu, K. T., A. H. Dutton, B. Geiger, and S. J. Singer. 1981. Ultrastructure of chicken cardiac muscle as studied by double immunolabeling in electron microscopy. *Proc. Natl. Acad. Sci. USA.* 78:7619–7623.
36. Singer, S. J., E. H. Ball, B. Geiger, and W. T. Chen. 1982. Immunolabeling studies of cytoskeletal associations in cultured cells. *Cold Spring Harbor Symp. Quant. Biol.* 46:303–316.
37. Avnur, Z., J. V. Small, and B. Geiger. 1983. Actin-independent association of vinculin with the cytoplasmic aspect of the plasma membrane in cell contact areas. *J. Cell Biol.* 96:1622–1630.
38. Otto, J. J. 1983. Detection of vinculin-binding proteins with an ^{125}I-vinculin gel overlay technique. *J. Cell Biol.* 97:1283–1287.
39. Jockusch, B. M., and G. Isenberg. 1981. Interaction of α-actinin and vinculin with actin: opposite effects on filament network formation. *Proc. Natl. Acad. Sci. USA.* 78:3005–3009.
40. Lin, S., J. A. Wilkins, D. H. Cribbs, M. Grumet, and D. C. Lin. 1982. Proteins and complexes that affect acting filament assembly and interactions. *Cold Spring Harbor Symp. Quant. Biol.* 46:625–632.
41. Vasiliev, J. M., and I. M. Gelfand. 1976. Effects of colcemid on morphogenetic processes and locomotion of fibroblasts. *In* Cell Motility. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory. 279–303.
42. DeBrabander, M., J. DeMey, R. Van de Veire, F. Aerts, and G. Geuens. 1977. Microtubules in mammalian cell shape and surface modulation: an alternative hypothesis. *Cell Biol. Int. Rep.* 1:453–461.
43. Albrecht-Buehler, G. 1979. Navigation of nonneuronal cells. *In* The Role of Intracellular Signals: Navigation, Encounter, Outcome. J. E. Nicholls, editor. Dahlem Konferenzen, Berlin. 75–96.
44. Albrecht-Buehler, G., and A. Bushnell. 1979. The orientation of centrioles in migrating 3T3 cells. *Exp. Cell Res.* 120:111–118.
45. Albrecht-Buehler, G. 1981. Does the geometric design of centrioles imply their function? *Cell Motility.* 1:237–245.
46. Vasiliev, J. M. 1982. Spreading and locomotion of tissue cells: factors controlling the distribution of pseudopodia. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 299:159–167.