Cell, Vol. 84, 359–369, February 9, 1996, Copyright ©1996 by Cell Press

Cell Migration: A Physically Integrated Molecular Process

Review

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Perspective

Cell migration plays a central role in a wide variety of biological phenomena. In embryogenesis, cellular migrations are a recurring theme in important morphogenic processes ranging from gastrulation to development of the nervous system. Migration remains prominent in the adult organism, in normal physiology as well as pathology. In the inflammatory response, for example, leukocytes immmigrate into areas of insult, where they mediate phagocytic and immune functions. Migration of fibroblasts and vascular endothelial cells is essential for wound healing. In metastasis, tumor cells migrate from the initial tumor mass into the circulatory system, which they subsequently leave and migrate into a new site. Finally, cell migration is crucial to technological applications such as tissue engineering, playing an essential role in colonization of biomaterials scaffolding.

As with many other cellular processes, the molecular components involved in cell migration are being identified at a rapid rate, and determination of how they participate in migration is following only somewhat more slowly. But also, like most other cell functions, the manner in which these components work together as a dynamic, integrated system to give rise to migration is only beginning to be studied. Understanding cell migration as an integrated process requires an appreciation of chemical and physical properties of multicomponent structures and assemblies, including their thermodynamic, kinetic, and mechanical characteristics, because migration is a process that is physically coordinated both spatially and temporally. Only when it is understood as an integrated system will its alteration via genetic, pharmacologic, or materials-based interventions acquire a truly rational basis.

In this article, we offer a perspective on cell migration emphasizing the physicochemical nature of underlying molecular mechanisms. Owing to imposed space and citation constraints, we focus on a limited set of issues, stressing conceptual insights. Readers interested in further discussions and literature citations are referred to some excellent reviews of relevant topics published in the past couple of years (Ginsberg et al., 1995; Hall, 1994; Huttenlocher et al., 1995; Janmey, 1994; Oliver et al., 1994; Schafer and Cooper, 1995; Sheetz, 1994; Stossel, 1993; Sun et al., 1995; Theriot, 1994). Here we examine, while focusing on their coordination, distinctive aspects of locomotion: morphological polarization, membrane extension, formation of cell–substratum attachments, contractile force and traction, and release of attachments. Almost all of the information we attempt to integrate comes from in vitro studies, mainly concerning movement across two-dimensional substrata. We nonetheless believe that much of the mechanistic understanding is relevant and useful for in vivo situations even in three dimensions. It is likely that cells interact with their surroundings by means of the same types of receptors in vivo as in vitro, and that physical interactions of cells with their environment play important roles in regulating function in both cases.

We further attempt to suggest generalizations across a wide spectrum of migratory cell types, including amoebae, leukocytes, fibroblasts, and neurons, looking for broad similarities among physical mechanisms. Observations of various cells demonstrating rapid, slow, or negligible locomotion on particular substrata may be explained as much by quantitative differences in physicochemical properties affecting how intracellular forces are generated and transmitted to the environment, using related processes, as by fundamentally distinct underlying mechanisms. Qualitative differences in migratory behavior may readily derive from quantitative differences in parameters that govern the integration of molecular components, altering relative balances of rates and forces. This is not surprising, since cell migration can be shifted between "on" and "off" by quantitative changes in the concentrations of molecular components (Huttenlocher et al., 1995), such as adhesion receptors, cytoskeletal-linking proteins, and extracellular matrix ligands. But cell migration can also be modified by quantitatively changing physicochemical properties such as receptor–ligand binding avidity (Duband et al., 1991) and strength of receptor–cytoskeleton interactions (Kassner et al., 1995). Thus, a productive view of cell migration, as well as other complex cell behavioral functions, will be that of a physically integrated molecular system in which changes in behavior are affected by quantitative alterations in the parameters characterizing kinetic and mechanical features of the molecular interactions.

Morphological Polarization

To migrate, cells must acquire a spatial asymmetry enabling them to turn intracellularly generated forces into net cell body translocation. One manifestation of this asymmetry is a polarized morphology, i.e., a clear distinction between cell front and rear. Concentration gradients of stimuli are not required to elicit this response. Polarization in macroscopically homogeneous stimulus environments may arise from perceived spatial or temporal stimulus gradients caused by microscopic nonuniformities or by kinetic fluctuations in receptor–ligand binding. An early event in polarization, at least for neutrophils, following stimulation of rounded cells by chemoattractant ligands is a change in filamentous, F-actin distribution from azimuthal symmetry around the cell rim to concentration at a particular region (Coates et al., 1992). Additional molecular rearrangements can ensue, leading to cellular spatial asymmetries involved in migration, such as forward redistribution of chemosensory signaling receptors (Sullivan et al., 1984), integrin adhesion

receptors (Lawson and Maxfield, 1995), and integrin–cytoskeleton linkages (Schmidt et al., 1993).

An important consequence of polarization is that extension of active membrane processes, including both lamellipodia and filopodia, takes place primarily around the cell front, so that directional turning is generally accomplished gradually, with cell locomotion taking on a persistent random walk character. The overall rate of cell migration in the absence of stimulus gradients is thus dependent on two independent quantities: linear cell locomotion speed and directional persistence time (Lauffenburger and Linderman, 1993). Molecular interventions can thus be usefully examined specifically in terms of changes in speed, persistence, or both. For instance, some alterations in integrin–cytoskeleton linkage influence migration speed but not persistence (Schmidt et al., 1995a). Variations among cell types in speed and persistence may also reflect differences in their spatial and temporal coordination of the various mechanisms involved in locomotion, such as force generation and adhesion. Over a spectrum of cell types, speed and persistence under optimal conditions appear to be inversely related, with slower-moving cells exhibiting greater persistence (Lauffenburger and Linderman, 1993), with the rapidly moving but highly persistent fish epidermal keratocyte serving as a notable exception. This relationship may reflect coordination between directional signaling and physical movement processes.

Membrane Extension

Lamellipodia are broad, flat, sheet-like structures, whereas filopodia are thin, cylindrical, needle-like projections. Cytoplasmic organelles are excluded from these structures, which abundantly contain actin and actin-associated proteins. Both can extend reversibly into three dimensions around the cell, even when the cell is crawling on a two-dimensional substratum. Actual speeds of cell translocation are not strongly correlated with the velocity of membrane protrusive flow (Condeelis, 1993), but a possible relationship between cell migration speed and the frequency of membrane extensions has not yet been rigorously examined.

Extension of both lamellipodia and filopodia in response to migratory stimuli is almost universally found coupled with local actin polymerization. Intervening details are complex and poorly understood (Condeelis, 1993; Stossel, 1993). An increase in the number of sites for actin polymerization is a first step, followed by net addition of monomeric, G-actin monomers to these F-actin growth sites predominantly near the membrane, in spite of fast turnover due to depolymerization. New open barbed-end sites for actin polymerization may arise by a combination of mechanisms, including uncapping of already-existing filaments, their severing, or both, as well as de novo formation of new actin trimeric nucleation sites (Sun et al., 1995; Theriot, 1994). In neutrophils, there is an increase in the total number of cortical actin filaments following chemosensory stimulation, without significantly altering the distribution of filament lengths (Cano et al., 1991), implying either that most increased polymerization occurs from new nucleation sites or that severing and uncapping occur concomitantly in coordinated tandem.

The gelsolin family is an attractive candidate for regulation of actin nucleation sites, because it regulates both severing and uncapping of actin filaments (Stossel, 1993). At micromolar and greater concentrations of calcium, and in the presence of low levels of the chemoattractant-induced phosphoinositides, the severing activity of gelsolin becomes significant, shortening filaments and increasing their number but leaving them capped. At less than micromolar concentrations of calcium, gelsolin dissociates from actin filaments, opening barbed ends for new polymerization. No discernable relationship has been found between calcium levels and membrane protrusion activity, however, so it is unclear whether these severing and uncapping activities are appropriately coordinated for promoting membrane extension (Condeelis, 1993). A modest correlation of gelsolin expression level with cell migration rate has been found in some studies (Cunningham et al., 1991; Witke et al., 1995) though not others (Andre et al., 1989; Cooper et al., 1987). It is possible that gelsolin activity may be important for aspects of cell locomotion other than lamellipod/filopod extension.

Uncapping of actin filament barbed ends would permit growth of existing filaments even in the absence of severing. Members of the calcium-independent capping protein family (Schafer and Cooper, 1995), such as capping protein β2, appear to be the barbed-end regulator of predominant importance in neutrophils (DiNubile et al., 1995) and perhaps other cell types as well. The time constant for F-actin recapping by capping protein is roughly a few seconds, consistent with the window needed to account for new actin polymerization kinetics. For new polymerization arising from uncapped F-actin barbed ends, however, there would be an increase in the filament length distribution and not in the number of filaments, in contrast with the findings of Cano et al. (1991). Although some nucleation activity by capping protein has been found in vitro, no such effect is noticeable in vivo, at least with Dictyostelium (Hug et al., 1995). As with gelsolin, a positive correlation of cell migration rate with capping protein expression level may result from an effect on actin cytoskeleton related to cell body translocation rather than membrane extension (Hug et al., 1995). Thus, the mechanism providing new actin polymerization sites for membrane extension in migrating cells remains unclear at present.

Instead, regulation of local free G-actin levels may be a primary effector for membrane extension. Whatever the number of uncapped growth sites, the amount of F-actin could potentially be increased by raising the concentration of G-actin monomer, which exists in two pools: free G-actin and G-actin bound to a monomer-binding protein. Indeed, were there no additional source pool of G-actin besides that existing as free monomer, the increase in the amount of F-actin due to uncapping all extant filament barbed ends would be negligible (Fechheimer and Zigmond, 1993). Three major families of cytoplasmic proteins that bind G-actin have been identified: β-thymosins, profilins, and ADFs/cofilins, each serving as a potential source of G-actin following release by migration stimuli (Sun et al., 1995; Theriot, 1994). However, the G-actin source effect of some of these proteins is negligible, while for others their function is more complex. Each of these families appears

to have a distinct role in controlling F-actin levels: β-thymosins as a G-actin source, profilins as a filament elongation promotor, and ADFs/cofilins as a filament cutter (Fechheimer and Zigmond, 1993; Sun et al., 1995; Theriot, 1994).

Theoretical analyses argue that local actin polymerization is in itself an adequate energy source for extension against the mechanical resistance provided by the cell membrane (Condeelis, 1993; Cooper, 1991), and experiments demonstrate that deformation of lipid vesicles occurs following induction of actin polymerization within the vesicle interior (Cortese et al., 1989). Moreover, a constant rate of new actin polymerization can lead to a constant rate of membrane extension, consistent with experimental observations, if viscous resistance by the membrane is not rate-limiting (Felder and Elson, 1990). Experimental evidence so far seems to weigh against a necessity for cell body contraction in membrane extension (Evans et al., 1993; Zhelev and Hochmuth, 1995), and perhaps for myosin motors more generally. Mutant cell lines defective in certain types of myosin molecules exhibit some defects in locomotion but not pseudopod extension (Titus et al., 1993; Wessels et al., 1988, 1991). Possible redundancies with alternative myosin isoforms are difficult to rule out, however. Localization of myosin I in membrane protrusions (Fukui et al., 1989; Yonemura and Pollard, 1992) could play a role in other aspects of migration, such as directed transport of adhesion receptors to enhance formation of attachments (Sheetz, 1994).

The ability of actin polymerization to drive membrane extension requires that actin filaments possess or aquire appropriate mechanical properties. In filopodia, actin filaments are grouped into rope-like bundles, while in lamellipodia they are cross-linked into lattice-like meshwork. Filament-binding proteins have begun to be classified according to their structures and activities; they include the fimbrin/α-actinin/filamin, villin, scruin, and fascin families (Matsudaira, 1994). Individual actin filaments can be bound by several different binding proteins simultaneously, permitting a diversity of organizational variations. Filament bundling and cross-linking both serve to increase the rigidity of the actin polymer network against the load of a membrane resisting deformation as a filopod or lamellipod attempts to extend. Thus, the activity of actin filament–binding proteins could be a key locus for regulation of membrane extension.

Consistent with this view, ABP-120, a member of the filamin subfamily, is required for normal rates of lamellipod extension in Dictyostelium (Cox et al., 1992), and similar findings have been obtained for a larger member of the same family, ABP-280, in melanoma cells (Cunningham et al., 1992). Importantly, subtle details of cross-linking structure may strongly affect membrane protrusion processes. For example, the number density of filaments in lamellipodia of cells lacking ABP-120 is at least as great as that in normal cells, but the spatial distribution is less regular and the interconnectedness is diminished (Cox et al., 1995). The lower extension rate could thus be due to a difference in mechanical properties of the cytoskeletal network. Moreover, lamellipodia and filopodia each contain physically connected growing filaments, but their extension rates and geometries are strikingly disparate. Both types of processes can be observed growing simultaneously in a single cell at the same location (Heidemann and Buxbaum, 1991), but they exhibit different distributions of actin-binding proteins yielding the different spatial structures (Matsudaira, 1994).

It is not clear whether the underlying physical growth mechanisms of lamellipodia and filopodia are identical, nor precisely what they are. Favored candidate mechanisms at this point in time, not mutually exclusive, are the Brownian ratchet (Peskin et al., 1993) and cortical expansion (Condeelis, 1993) models. In the Brownian ratchet mechanism, actin monomers may be added to filaments proximal to the cell membrane when thermal fluctuations of the membrane position allow the requisite room. In the cortical expansion mechanism, the actin filament gel is proposed to swell from local influx of water either due to increased osmotic potential, possibly resulting from filament severing, or more generally due to an entropic driving force when filament–water interactions are energetically favorable. Relative contributions of the Brownian ratchet mechanism versus the corticol expansion mechanism could be favored in filopodia and lamellipodia, respectively, on the basis of their comparative structures of a highly oriented tight bundle versus a looser network mesh.

Formation and Stabilization of Attachments

Along with a bias for membrane extension at the cell front, there may also be a preferential ability of attachments to form at the leading edge of lamellipodia and filopodia. Several observations point to the cell front as a preferential locus where adhesions form. Interference reflection microscopy (IRM) images of migrating heart fibroblasts show new focal adhesions forming at the cell front and persisting until they reach the cell rear (Izzard and Lochner, 1980). Video tracking of integrins using non-adhesion-perturbing antibodies directed against β1 integrins also reveals the front as a site where new adhesions tend to form (Regen and Horwitz, 1992). Nascent adhesions appear in temporal waves, initially as small aggregates that trace the geometry of the leading lamella. These aggregates increase in size and intensity as the cell migrates over them, persisting and remaining fixed on the substratum until they reach the rear, or an edge, of the cell. While specific molecules that initiate or nucleate the formation of adhesive complexes have not been identified, some evidence points to the existence of a preformed cytoskeletal complex that precedes the incorporation of adhesion molecules. IRM studies, for example, demonstrate that development of actin filament stress fibers precedes the formation of focal adhesions (Izzard, 1988).

Cell–substratum attachments at the leading edge that subsequently remain fixed to the substratum as the cell moves forward effectively serve to remove adhesion molecules from the leading lamella. This implies existence of mechanisms to replenish such components at the cell front. Such a process has been demonstrated with tracking of gold aggregates conjugated to reagents directed against cell surface proteins. These studies

demonstrate that membrane proteins, including integrins, are directed rapidly toward the cell periphery, including the leading edge, where they tend to remain (Schmidt et al., 1993). Increased concentrations of other cytoskeletally associated components are also enriched in the leading lamella, although the mechanism of their recruitment is not known (Nobes and Hall, 1995; Wu and Goldberg, 1993). In addition to this directed surface movement of adhesion receptors, vesicle trafficking of adhesive components to the leading edge continues to be proposed as a replenishing mechanism (Bretscher, 1992); it remains an intriguing hypothesis, although direct data in its favor are elusive (Lawson and Maxfield, 1995).

Mechanisms that nucleate and regulate the organization of adhesive complexes are currently under intense study. Covalent modification of proteins by tyrosine phosphorylation is strongly implicated in the formation of adhesive structures. Upon adhesion to a substratum or the extension of a filopodium, a group of cytoskeletal associated proteins are phosphorylated on tyrosine. Focal adhesion kinase (FAK), paxillin, and tensin are among the prominent and best characterized of these phosphoproteins that comprise adhesive complexes (Lo et al., 1994; Schaller and Parsons, 1994; Turner, 1994).

While there is no convincing evidence at present demonstrating that either FAK, tensin, or paxillin plays a critical role in the formation of adhesive complexes, these molecules are leading candidates. They are among the earliest adhesive proteins that undergo phosphorylation, and phosphoproteins, including FAK, are present in nascent adhesions (Wu and Goldberg, 1993). Further, inhibitors of tyrosine phosphorylation inhibit their phosphorylation and correspondingly diminish cell migration and spreading (Burridge et al., 1992). A mechanism through which these molecules contribute to the formation of adhesive complexes remains to be clarified, but tyrosine phosphorylation of paxillin, tensin, and FAK creates in each case recognition sites for proteins containing src-homology 2 (SH2) domains. These sites, along with their other binding sites, likely play key roles in the assembly of adhesive complexes. For example, FAK has binding sites via its SH2-binding domain for src-related tyrosine kinases, including fyn, csk, and src; it also has binding sites for structural proteins such as paxillin, talin, and integrin (Schaller and Parsons, 1994; Chen and Guan, 1994; Chen et al., 1995). In addition to its binding properties, FAK exhibits tyrosine kinase activity and phosphorylates cytoskeletal-associated substrates such as src and paxillin, which in turn could initiate further recruitment of structural and signaling components (Schaller and Parsons, 1995). Likewise, paxillin has numerous SH2-binding domains, an SH3-binding domain, and LIM domains (Turner and Miller, 1994). It binds structural components such as vinculin as well as signaling molecules, including src, csk, FAK, and crk (Schaller and Parsons, 1995; Turner, 1994). Finally, tensin binds to src and paxillin through its SH2 domain, while through other domains it binds to actin and SH2 domain–containing proteins (Davis et al., 1991; Lo et al., 1994). Thus, through these multiple binding domain proteins, molecular interconnections can be enhanced, modified, and linked to signaling pathways. The physical properties of these interconnections, such as their response to and ability to transmit mechanical forces, are almost certainly governed by signal-induced chemical modifications and are likely to be a key aspect of regulation of migration; this point will be discussed in following sections.

In contrast with the uncertainty regarding the mechanisms by which adhesive complexes form, rapid progress is being made on identifying the molecules that regulate their formation. There is convincing evidence that cdc42, rac, and rho—all members of the rho subfamily of the ras family of GTP-binding proteins—play a major role in regulating the formation of adhesions. These regulatory proteins comprise a hierarchical cascade that initiates the formation of filopodia, lamellipodia, and focal adhesions and stress fibers (Hall, 1994). Formation of filopodia is regulated by cdc42 (Kozma et al., 1995; Nobes and Hall, 1995), while formation of lamellipodia is regulated by rac, whose activation stimulates membrane ruffling and the formation of lamellipodia (Ridley et al., 1992). Finally, formation of focal adhesions, highly organized adhesive complexes containing termini of actin stress fibers, is regulated by rho (Ridley and Hall, 1992). Activation of cdc42 initiates this cascade (Nobes and Hall, 1995).

Although cdc42, rac, and rho are involved in processes relevant to cell locomotion, the mechanisms through which they exert their effects are not known. Because filopodia, lamellipodia, and focal adhesions each involve distinct organizations of F-actin, the actions of this GTP-binding subfamily could be fairly proximal to regulation of actin-binding proteins. Candidates for intermediary actors are tyrosine kinases, including FAK; lipid kinases, including phosphatidylinositol phosphate (PIP) 5- and phosphatidylinositol (PI) 3-kinases; and phospholipase $C\gamma$ ($PLC\gamma$). Each of these is implicated in migration in a characteristic way: FAK, as discussed above, is thought to initiate formation of adhesions; PIP 5-kinase makes PIP_2, which is implicated in the assembly of actin filaments; and PI 3-kinase is implicated in chemotactic responses and modulation of integrin affinity (Hartwig et al., 1995; Kovacsovics et al., 1995; Kundra et al., 1994; Wennstrom et al., 1994). $PLC\gamma$ is required for PDGF-, IGF-1-, and EGF-induced migration (Bornfeldt et al., 1994; Chen et al., 1994; Kundra et al., 1994), presumably via hydrolysis of PIP_2 and mobilization of actin-binding proteins.

Contractile Forces and Traction

At least two distinct types of force must be generated independently by a locomoting cell. The first is the protrusive force needed to extend membrane processes, lamellipodia or filopodia. As discussed earlier, actin polymerization and structural organization by means of cross-linking into lattices or bundles, respectively, likely provide this force independent of myosin motor activity. The second force is a contractile force, needed to move the cell body forward. This force appears to depend on active myosin-based motors and may in fact involve separate mechanisms of force generation within the anterior and posterior regions of the cell. Clearly, the phenomena of extension and contraction—while coordinated (Chen, 1981)—can occur independently of one

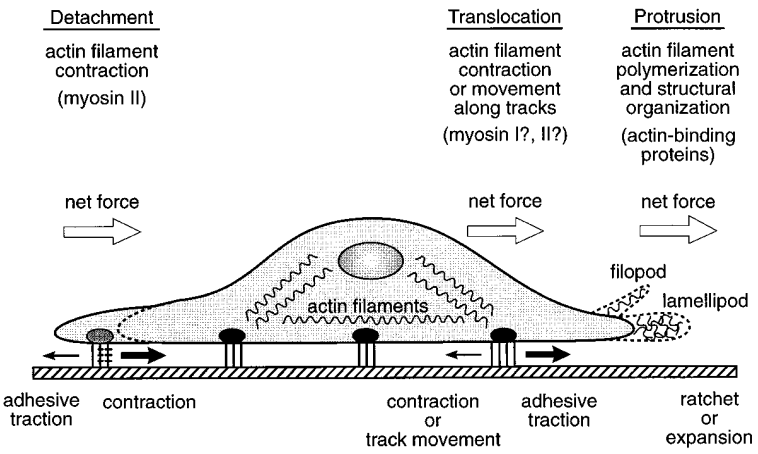

Figure 1. Illustration of Different Forces Involved in Cell Migration

Protrusion of membrane lamellipodia or filopodia requires force generated by actin polymerization, by the Brownian ratchet mechanism, the cortical expansion mechanism, or a combination of these. Translocation of the cell body forward, once the membrane protrusion has become adherent to the substratum, may occur by myosin interactions with actin filaments; possible mechanisms for this include contraction of filaments connecting cell–substratum adhesion complexes with intracellular structures, or relative movement of adhesion complexes across cortical actin filament "tracks." In either case, the magnitude of traction is greater than the rearward pull on the adhesion complexes. Detachment of the cell rear involves disruption of cell–substratum attachments, perhaps accelerated by myosin-mediated actin filament contraction pulling on adhesion complexes. Here, the magnitude of traction is less than the contraction force.

another (Evans et al., 1993; Jay et al., 1995; Marks et al., 1991) and may use different mechanisms to generate their respective appropriate forces. Figure 1 illustrates a plausible working view of these various forces. The actual traction exerted by a cell on its substratum is directly related, but not identical, to the intracellularly generated contractile force, because these can be dissipated by deformation of the cell and by disruption of cell–substratum attachments. In turn, the substratum locally exerts an equal and opposite traction force on the cell via the same attachments, with magnitude depending on the susceptibility of the attachments to disruption.

Hence, the magnitude of cell-generated contractile forces does not by itself determine cell migration speed. Theoretical analyses predict that the ratio of contractile force to cell–substratum adhesion strength contributes to the rate of locomotion in a biphasic manner, with maximal migration speed associated with an intermediate ratio of force to adhesiveness (DiMilla et al., 1991), and some experimental data are consistent with this prediction (DiMilla et al., 1993; Keely et al., 1995).

One can infer from a compilation of existing data that maximal cell migration speed tends to correlate inversely with contractile force (Oliver et al., 1994). Connective tissue fibroblasts typically generate a much stronger traction than do fish epidermal keratocytes, but the latter migrate much more rapidly. The effective traction generated by fibroblasts has been measured to be approximately 2×10^4 μdynes (Harris et al., 1980), whereas that generated by keratocytes is about 2×10^3 μdynes (Lee et al., 1994); maximal migration speeds of these two cell types are on the order of 1 μm/min for fibroblasts and 10 μm/min for keratocytes. Neutrophils exert an intermediate level of force (Evans et al., 1993) and exhibit maximal migration speeds intermediate between fibroblasts and keratocytes. According to the theoretical analysis of DiMilla et al. (1991), then, these observations would imply that the optimal cell–substratum adhesive strengths yielding maximal migration speeds for fibroblasts, neutrophils, and keratocytes, respectively, would be in descending order, with approximately 10-fold interval decreases. A small amount of data gathered to date is consistent with this trend qualitatively, though with not quite as strong a decrease (Oliver et al., 1994). Differences among these cell types in the vectorial directions of contraction and traction (Harris et al., 1980; Lee et al., 1994; Simon and Schmid-Schoenbein, 1990) could also influence their maximal migration speeds.

The resistance that contractile forces must overcome in order to accomplish cell body translocation is primarily due to adhesive interactions. Viscous drag by the fluid medium has been calculated to be less than 0.1 μdyne, a value that is small relative to the cell contractile force (Oliver et al., 1994), and nonspecific so-called solid friction between cell and substratum is similarly negligible. Thus, effective contractile force must essentially be in balance with the traction force provided by dynamic cell–substratum attachments in order to move the cell body (Huttenlocher et al., 1995). Moreover, this balance must incorporate an asymmetry in traction between the cell front and rear, allowing forward attachments to remain while rearward attachments are released (Sheetz, 1994). The degree of this asymmetry is predicted to be a key determinant of the range of substratum ligand concentrations permitting locomotion; the greater the asymmetry, the wider the range of permissive concentrations (DiMilla et al., 1991).

This dependence of cell locomotion speed on overall cell–substratum adhesive strength and the degree of spatial asymmetry in adhesive strength suggests one means by which the various molecules regulating adhesion complexes, as described in the previous section, can effectively control migration. The mechanical strength of protein–protein bonds is logarithmically related to their biochemical affinities (Kuo and Lauffenburger, 1993), so alteration of the affinities of linkages within adhesion complexes, by covalent modifications, can "tune" overall adhesiveness as well as a spatial adhesiveness differential.

The roles of myosin motors in generating intracellular contractile forces have been studied intensely in Dictyostelium and to a lesser degree in fibroblasts (Wolenski, 1995). Myosin II is a double-headed, long rod-like molecule, capable of polymerizing into bipolar filaments, whereas myosin I is a single-headed molecule with only a short tail. Both can bind to actin filaments and produce ATP-dependent motion. Myosin II is abundant in the cell cortex, and its bipolar filaments can pull two actin filaments past one another as in muscle contraction. A number of myosin I subtypes exist possessing variations in their tails, enabling movement of bound entities such as vesicles, membrane-associated proteins, or other actin filaments along an actin filament.

Commensurate with its spatial distribution and structural organization, myosin II can produce a cortical tension causing Dictyostelium cells to round up and detach from a surface (Pasternak et al., 1989). Myosin II–deficient amoebae crawl more slowly than wild-type parental strains, despite extending pseudopodia in an apparently normal fashion (Wessels et al., 1988). This effect is exaggerated on highly adhesive substrata (Jay et al., 1995). Transport of beads attached to the membrane at the cell rear is slower at the uropodal rear of myosin II–deficient cells, and formation of ultrathin lamella at the cell tail is diminished there as well, both suggesting that the contractile force generated by myosin II acts strongly at the uropod (Jay et al., 1995; Jay and Elson, 1992). This concept is consistent with the spatial distribution of myosin II found in migrating cells: it is highly concentrated in posterior regions in Dictyostelium (Rubino et al., 1984; Yumura et al., 1984) and more mildly so in 3T3 fibroblasts (Conrad et al., 1993).

A major function of myosin II–based contraction in migrating cells, then, may be to help break adhesive interactions by direct application of physical stress (Jay et al., 1995). Addition of ATP to a permeabilized cell system results in focal adhesion breakdown in a myosin-dependent manner (Crowley and Horwitz, 1995), suggesting the active involvement of myosin-generated force. Myosin II–mediated contraction of actin filaments could pull on filaments connected to integrin adhesion receptors that are linked in turn to extracellular matrix ligands. This input of force could potentially accelerate bond disruption, either at the extracellular receptor–ligand site or at an intracellular receptor–cytoskeleton site, depending on which connection is most labile.

This role for myosin II–based contraction is consistent with its stimulation, in turn, by intracellular calcium concentrations, and perhaps provides a most important role for calcium in cell migration. Transient increases in intracellular calcium that occur during neutrophil migration regulate integrin-mediated adhesion to some extracellular matrix ligands and are required for detachment of the cell rear and effective migration (Maxfield, 1993). Moreover, calcium concentrations are lowest at the leading edge and highest in the posterior regions of migrating leukocytes (Hahn et al., 1992), so that myosin II–based contraction would be greatest at the cell rear, where release of cell–substratum attachments must occur.

Myosin-based contractile forces may also be generated behind the leading lamella but in front of the nucleus. Large beads bound to integrin aggregates are pulled rearward toward the nuclear region in a manner dependent on integrin–cytoskeleton interactions (Schmidt et al., 1993). A rearward-directed force is exerted anterior to the nucleus even in myosin II–deficient Dictyostelium cells (Jay and Elson, 1992), and myosin II–deficient cells are able to locomote, albeit more slowly than wild-type (Jay et al., 1995; Wessels et al., 1988). Thus, either myosin II or myosin I could serve to pull the cell body forward by contractile forces acting between the back edge of the leading lamella and the nucleus, while myosin II contraction at the cell rear reduces the resistive cell–substratum traction. The forward pull could be due to connections between integrin–extracellular matrix linkages and some structure(s) physically associated with the nuclear endoplasm, according to an endoplasmic contraction mechanism (Sheetz, 1994). Deletion of myosin II reduces rearward bead motion, and correspondingly contraction of the cortical actin cytoskeleton, more severely than it reduces cell migration speed (Jay et al., 1995; Jay and Elson, 1992; Wessels et al., 1988). Cortical actin appears to remain relatively fixed with respect to the substratum frame of reference in fast-moving keratocytes (Theriot and Mitchison, 1991), perhaps providing a "track" on which intracellular structures can move (Bilozur and Hay, 1989; Sheetz, 1994). In slower-moving fibroblasts, cortical actin "flows" rearward at a noticeable rate (Wang, 1987), perhaps diminishing the traction able to support forward movement of the cell body. Consistent with this concept are experiments showing that the locomotion speed of nerve growth cones is inversely proportional to the rate of retrograde actin flow (Lin and Forscher, 1995).

Rear Release

Rapid migration requires efficient mechanisms to release adhesions at the rear of the cell. While rates of lamellipodal protrusion and rear release both potentially contribute to the migration rate, in some cells the rate of rear release determines the overall migration rate (Chen, 1981). Fates of adhesion molecules, as revealed by tracking using low light video microscopy with antibodies conjugated with fluorescent (or photoactivatable fluorescent) derivatives, provide insights about release mechanisms as well as receptor fate following release of adhesions. Tracking studies of β1 integrins on migrating fibroblasts reveal that the major fraction of integrins are left on the substratum as the cell releases and moves forward (Regen and Horwitz, 1992). This membrane "ripping" has been reported previously both in vitro and in vivo (Bard and Hay, 1975; Chen, 1981). Cytoskeletally associated molecules such as talin and vinculin are not present in these membrane remnants, but rather, they tend to accumulate at the rear of the retracted cell. Although ripping appears to be a major mechanism for release for fibroblasts, it has not been described in other cell types.

Integrins remaining on the cell surface undergo two fates: a regulated release in which they disperse on the cell surface and endocytosis into vesicles that accumulate in the cell body (Palecek et al., 1996; Regen and

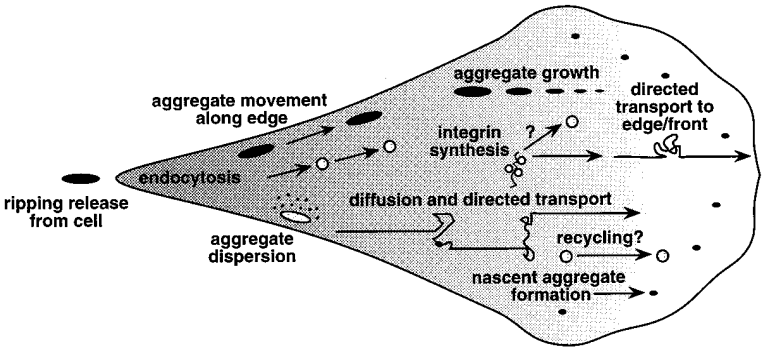

Figure 2. Illustration of Integrin Adhesion Receptor Dynamics in Cell Migration

Newly synthesized or recycled integrins may be inserted into the plasma membrane behind the leading lamella, then transported to the edge by diffusion and directed transport (possibly mediated by myosin I actions). Once there, adhesion bonds are formed with substratum ligands, followed by nascent aggregates—adhesion complexes—which grow as the cell moves forward. At the rear of the cell, these adhesions are released as the trailing edge detaches from the substratum. Their integrin contents may be extracted from the membrane and left behind on the substratum, or dispersed by diffusion on the membrane surface, or internalized. The aggregates may also move along the edge of the migrating cell for a while before being eventually disrupted.

Horwitz, 1992). The cell edge, particularly at the cell rear, is a locus at which adhesive aggregates tend to release. Once there, they either release and disperse to be used for new adhesions toward the cell front or, alternatively, remain aggregated, move forward along the cell edge, and form a new adhesion. Another fraction of the integrins from rear adhesions appear in endocytic vesicles that accumulate in the perinuclear region.

The loss of cell surface integrins by the ripping mode of release and by endocytosis points to a need for mechanisms by which integrins are replenished on the cell surface. The loss of integrins in tracks that remain on the substratum behind the cell points further to a need for continued biosynthesis. The location of this biosynthesis and the mechanism of its trafficking to the leading lamella are not known. mRNA transcripts for an actin isoform are targeted to the leading lamella (Latham et al., 1994), so perhaps the mRNAs for other, adhesion-related molecules could be similarly targeted.

Recycling through endocytic vesicles is another possible route by which integrins accumulated in the rear might be supplied to the cell front (Bretscher, 1992). In contrast with the cell rear, however, significant numbers of vesicles have not been reported near the leading edge, where they might provide a supply of receptor for new adhesions. It is more likely that the vesicles appear at the surface elsewhere in the cell and their contents subsequently move forward by diffusive transport. Directed motions, such as those observed on the cell front (Schmidt et al., 1993), may also transport integrins on the surface from the rear to the front. Figure 2 shows an overall view of integrin dynamics across a migrating cell.

In accord with observations that adhesions tend to release at the rear, the cell rear is a region where integrin–cytoskeletal linkages tend not to form and the membrane is less well-supported by the cytoskeleton. This contrasts with the cell front, where integrin–cytoskeletal linkages do tend to form, and the membrane is well-supported. Optical trapping observations using integrin antibodies show that migrating fibroblasts are highly polarized, demonstrating at least a 4-fold difference between the front and rear in their ability to form cytoskeletal linkages (Schmidt et al., 1993). An analogous asymmetry is seen in migrating neuronal growth cones (Schmidt et al., 1995b). The cell rear is further distinguished from the cell front by its tendency to have unsupported membrane resulting in the formation of membrane tethers, i.e., regions of membrane—depleted of cytoskeletal components—that can be pulled out from the cell (Schmidt et al., 1993, 1995b).

The mechanisms by which adhesions release in the rear likely result from the combination of several, possibly unrelated, mechanisms. These include a mechanical contribution arising from cytoskeletal contraction, and contributions from signaling pathways involving regulatory components such as rho, calcineurin, and tyrosine kinases. Increasing evidence points to cytoskeletal tension, or contraction, as a mechanism by which adhesive complexes break down as well as organize. Myosin II mutants of Dictyostelium show inhibited migration on more adhesive substrates (Jay et al., 1995). Furthermore, injection of antibodies against myosin light chain kinase into macrophages inhibits their migration (Wilson et al., 1991). Studies using a permeabilized cell system that stabilizes adhesive complexes also point to a contribution from cytoskeletal contraction to adhesive stability (Crowley and Horwitz, 1995). Addition of ATP to permeabilized cells, under certain conditions, stimulates cytoskeletal contraction and a breakdown and reorganization of adhesive components. Peptides that inhibit the actin–myosin interactions also inhibit the breakdown of adhesive complexes. While this mechanism is operative in fibroblasts, its presence in other cells remains to be investigated. Because of the dependence of linkage strength on affinity (Kuo and Lauffenburger, 1993), relative breakage labilities of alternative sites may be influenced by covalent modifications that alter linkage affinities, permitting biochemical control of physically induced disruption.

Hence, there is motivation for components of signal transduction pathways being involved in regulation of rear release mechanisms. The GTP-binding protein rho is strongly implicated in adhesive release; inactivation of rho by either inhibitors or physiologic regulators induces cytoskeletal breakdown, cell rounding, and inhibited migration in several different cell types (Miura et al., 1993; Paterson et al., 1990). Calcineurin, a calcium/calmodulin-regulated serine/threonine phosphatase, plays a key

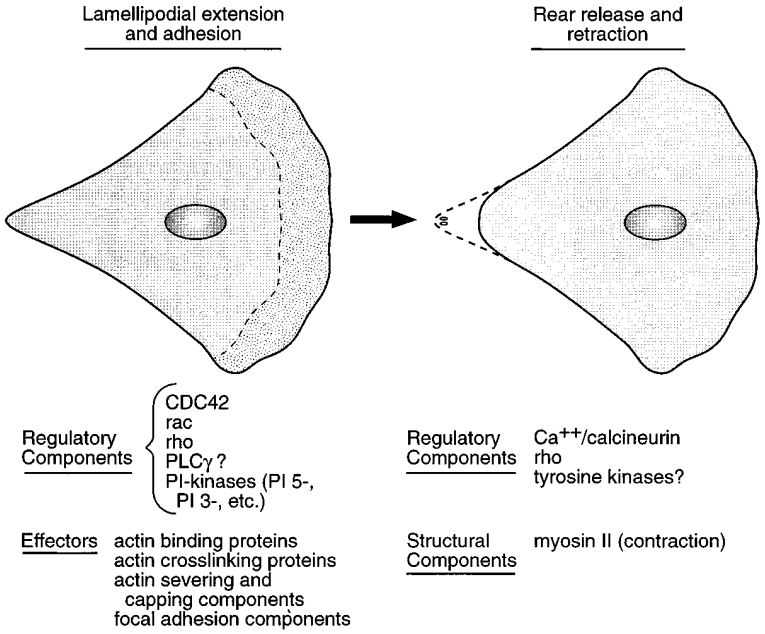

Figure 3. Components Implicated in Cell Migration

Compilation of regulatory and effector components apparently implicated in cell migration mechanisms of membrane protrusion and attachment to the substratum, and release of the cell rear followed by retraction of the tail.

role in the release of cells adherent to vitronectin (Hendey et al., 1992). Addition of a peptide inhibitor of calcineurin to neutrophils inhibits their migration, and video microscopy of these cells reveals repeated protrusion of the leading lamella but inhibited detachment from the substratum.

Several observations point to a role for tyrosine phosphorylation in detachment. Cells transformed with viruses expressing oncogenes encoding tyrosine kinases, such as pp60^{src}, tend to appear more round and less adherent and to show enhanced migration (Burridge et al., 1988). The addition of ATP to permeabilized cells provides additional, direct evidence for a role for tyrosine phosphorylation. Under some conditions, ATP treatment stimulates the tyrosine phosphorylation of several major proteins and induces breakdown of adhesions. Both the phosphorylation and reorganization of adhesive components are inhibited by addition of an exogenous, recombinant tyrosine phosphatase (Crowley and Horwitz, 1995).

Overall Coordination

Although understanding of individual processes underlying cell migration continues to increase, information concerning how they are coordinated spatially and temporally remains limited. Both chemical and physical mechanisms for global regulation almost certainly act in concert. The relationship between bond affinity and strength should allow changes in adhesiveness and force transmission to result from biochemical modifications of proteins, motivating a connection between biochemical signal transduction pathways and physical determinants of locomotion. Figure 3 illustrates key components in both aspects of migration.

The possibility is appealing that spatial concentrations, or localized temporal concentration transients, of second messengers such as calcium and phosphoinositides—or of enzymes and motor proteins whose activities they regulate—are distributed differentially across the cell length. As one simple possibility, high calcium concentrations at the cell rear would activate proteins that disrupt actin filament networks (Janmey, 1994) and would enhance myosin II contractile activity to promote release of attachments there (Conrad et al., 1993; Maxfield, 1993; Jay et al., 1995). Low calcium concentrations and high phosphoinositide levels at the cell front would activate proteins that cross-link actin filaments, facilitating membrane extension (Janmey, 1994); myosin I activity favored by low calcium levels at the cell front (Conrad et al., 1993) might additionally permit directed membrane–protein transport activity to promote formation of new attachments (Schmidt et al., 1993), or contraction to pull cell body structures forward (Sheetz, 1994), or both. Findings concerning spatial distributions of second messengers themselves are often inconsistent (e.g., Laffafian and Hallett, 1995), so it may be that the regulated proteins are more particularly localized.

Covalent modification activities of numerous kinases and phosphatases on a variety of signaling and structural proteins, especially those organized in adhesive complexes linking adhesion receptors to the actin cytoskeleton, are almost certainly spatially distributed as well (Huttenlocher et al., 1995). This could regulate a front-versus-rear asymmetry in cell–substratum traction by altering lability and force transmission capability of the adhesive complexes.

Concomitantly distributed mechanical stresses could simultaneously provide for globally coordinated influence, because tension can suppress membrane protrusion perpendicular to the axis of tension, possibly by promoting actin filament disassembly (Kolega, 1986) or by stress-induced alterations in activity of enzymes associated with the cytoskeleton. Cytoplasmic strains and substrate tractions have been examined in detail for keratocytes and neutrophils, respectively, and found to vary significantly in magnitude and direction around the cell (Lee et al., 1994; Simon and Schmid-Schoenbein, 1990). Microtubules, perhaps conspicuous by their absence from our discussion until this end, are more likely

to play a role in physical coordination than in the individual processes of force generation or substratum interactions.

This view of cell migration as a spatially and temporally integrated process likely pertains to other cellular functions as well. Gene regulation through structural alteration in nuclear and chromatin structure (Boudreau et al., 1995) and cytoskeleton–extracellular matrix interactions that propagate to the nucleus (Schwartz and Ingber, 1994) are converging on a similar theme. The next decade should see significant progress, and certainly some surprises, in our understanding of integrated cell behaviors, including signal transduction, growth, and differentiation.

Acknowledgments

The authors are grateful for financial support from the National Institutes of Health (grants GM23244 and GM53905), and to very helpful comments on the manuscript from Elliot Elson, Vlodya Gelfand, Paul Matsudaira, Julie Theriot, and Sally Zigmond. D. A. L. and A. F. H. would also like to thank Alan Wells, and Anna Huttenlocher and Rebecca Sandborg, respectively, for stimulating conversations on this subject, and Sean Palecek for Figure 2. Finally, we extend our apologies to all our colleagues in the field whose work we were unable to cite formally because of imposed reference limitations.

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