Cell Locomotion

Secondary article

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The crawling locomotion of animal cells results from a coordinated cycle of protrusion, attachment and retraction. Protrusions in the direction of motion are normally generated by controlled assembly of actin networks, while adhesion and retraction rely as well on tension generated by actin–myosin interactions. Microtubules control the spatial distribution of these activities, creating a polarized shape in the cell that determines the direction of motion. All these processes are coordinated by small G proteins of the Rho family.

Article Contents

Paradigm of Crawling Locomotion: Protrusion, Adhesion and Retraction

Cells of many types exhibit a crawling locomotion by which they are able to move while attached to a surface (**Figure 1**). This phenomenon, which has been studied for many years in highly motile examples such as amoebae, exists to some extent among nearly all types of animal cells. Locomotion is of course crucial for amoebae that must move to find food and to avoid harmful products. They normally crawl along chemotactic stimuli. In multicellular organisms, cell locomotion is required mainly in development, and in wound healing. Escaping the normal regulation on locomotion, cancer cells are able to invade healthy tissues and to form tumours there.

Some cells are particularly efficient movers. This may relate to their specialized roles *in vivo*. Leucocytes seek out and invade tissues that display an alarm response, and attempt to neutralize the danger as a first stage of the immune response. Keratocytes from fish scales and amphibian skin move very elegantly when explanted in culture. Fibroblasts and epithelial cells are normally stationary but move when stimulated in wound healing. Sperm cells in the majority of species move by a swimming motion. The sperm of nematodes represents an exception; these cells move by fast crawling.

During development, some cell types migrate to very long distances, particularly neural crest cells. Precursors of neurons are themselves quite motile, and this feature is essential to achieve the complex architecture of the nervous system. Once the cell body finds its ultimate location, it can send out long extensions as axons and dendrites that branch and connect with other cells throughout the body. The leading part of the axon, called the axonal growth cone, moves by a crawling locomotion very similar to that of individual motile cells.

The motility of all crawling cells has a common feature in the polarized formation of cytoplasmic protrusions that determine the direction of motion, called pseudopods in the most general sense (**Figure 2**). These extensions may be produced by fluid pressure, where contraction of the cell

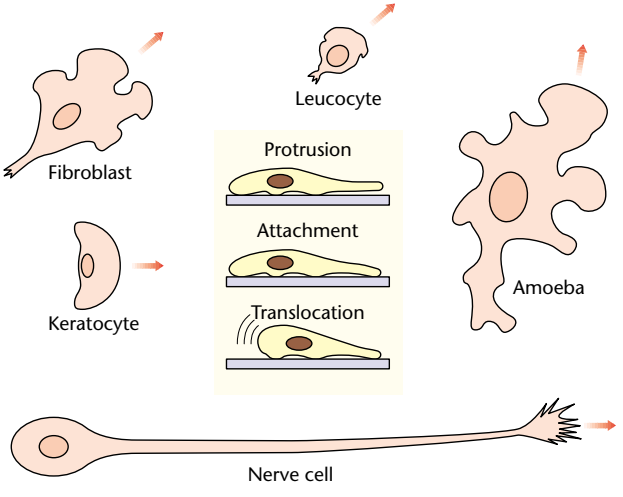

Figure 1 Adherent cells crawl along surfaces by extending protrusions (pseudopods) in the direction of motion, as shown in the central panel. These protrusions attach to the substrate, and then the cell draws its body forward. In many cases this forward movement is driven by internal contractions, followed by release of the rearmost attachments. The cycle repeats with the extension of new pseudopods. Different cell types show a variety of mechanisms. The velocity, persistence of directionality, and morphology of cells differ widely. Shown are several examples of extensively studied motile cell types. Keratocytes move rapidly while maintaining a constant half-moon shape. *Amoeba proteus* is a traditional model for amoeboid movement; the mechanism of protrusion formation is quite different from that in most tissue cells. Crawling fibroblasts and leucocytes differ in their degree of attachment to the substrate, and in their typical velocities. Fibroblasts move slowly while applying forces to the extracellular matrix much larger than those required for movement, while leucocytes are weakly attached to the substrate and move more rapidly. Nerve cells do not translocate their bodies, but they extend long processes by means of growth cones at the tips that behave very much like motile cells.

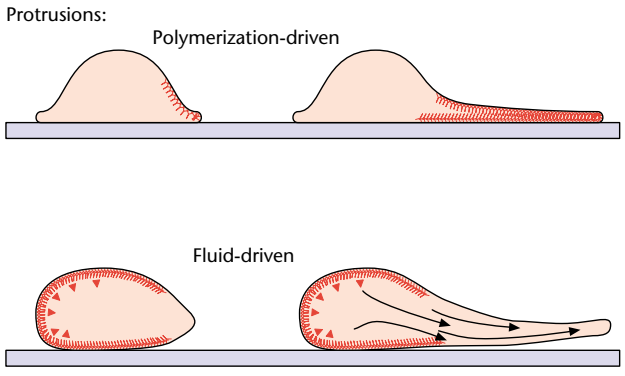

Figure 2 Two mechanisms for the formation of pseudopods are shown. They are probably extreme idealizations of what would normally be a mixture of the two. The mechanism most intensively studied in recent years is based on polymerization of cytoskeletal filaments, normally actin, within the lipid plasma membrane envelope. In the case of nematode sperm cells, protrusion is driven instead by polymerization of a protein known as major sperm protein (MSP). Another mechanism is based on the effect of fluid pressure created by contraction of the cell cortex, which forces the membrane to bulge in a direction where the cortex is weakened. While this classic model is most suggestive in amoebae, for which it was first proposed, it may operate to some extent in other cell types as well.

membrane in the rear squeezes the intracellular medium into a bubble of the plasma membrane that protrudes from the cell body. Alternatively, protrusions may be produced by polymerization of solid filaments within the cell that press on the plasma membrane from within. In this case the most prevalent morphologies are known as lamellipodia and filopodia. As the names imply, the former are flat, filled with a diagonally-linked filament network, while the latter is tubular and contains a bundle of parallel filaments. It is likely that the relative importance of these two mechanisms depends on the degree to which a particular cell type adheres to a surface. The fluid mechanism may prevail in amoeba-like cells, while the polymerization mechanism would be more effective in tightly-attached tissue cells as they crawl along a surface. This article focuses on the latter type of crawling movement. In both cases, protrusion is followed by attachment of the pseudopod to the substrate, followed by movement of the cell body, release of the rearmost attachments, and retraction of the rear of the cell. Each of these processes deserves a special discussion. The diversity of crawling behaviours comes largely from different dependence on each stage. The maintenance of directionality is also an important factor in locomotion, as well as another source of diversity among cell types.

Formation of Actin-filled Protrusions at the Leading edge

Lamellipodia and filopodia are membrane extensions filled by filaments of cytoskeletal proteins. These proteins are transformed from a monomeric form into an extended network of branching or crosslinked polymers, in the case of lamellipodia, or parallel bundles in the case of filopodia (**Figure 3a**). With one known exception to be discussed later, this protein is actin. Much has been learned about the mechanisms of such protrusion formation by studying the intracellular movements of bacteria, such as *Listeria* and *Shigella*, that propel themselves forward by polymerizing a tail of actin to the rear. When these bacteria approach cell membrane, they can produce membrane extensions and even protrude through the membrane (**Figure 3b**). What is the origin of the force that deforms the membrane? A popular theoretical model suggests that the polymerization itself may provide sufficient force by a so-called elastic Brownian ratchet mechanism. Thermal undulations of the filaments create gaps between their tips and the membrane, allowing sufficient space for addition of new actin monomers. These extend the filaments and press the membrane forward as they return (**Figure 4**). Entirely *in vitro*, sealed lipid vesicles can be deformed by polymerization of cytoskeletal filaments within. While such experiments show that membrane extension by a ratchet mechanism is possible, other elements in the cell are likely to contribute. Particularly important is the balance of lipid flow, as the cell maintains a constant turnover of its membrane via uptake and fusion of small vesicles. Their delivery may be biased is such a way as to supply extra membrane to the extending region (Bretscher and Aguado-Velasco, 1998).

Other than actin itself, essential components for this extension are proteins that regulate the polymerization and generation of branched structures, proteins that maintain

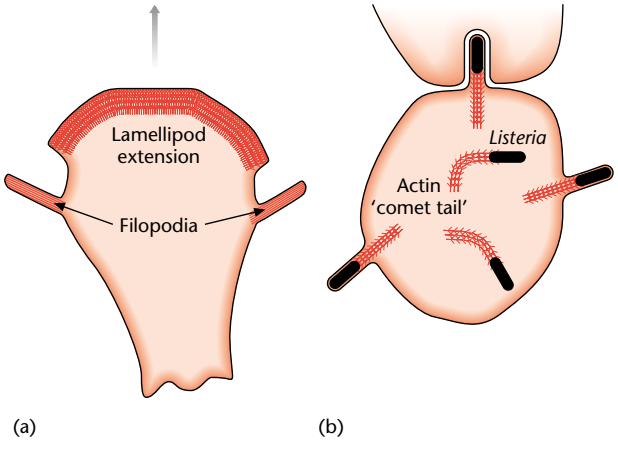

Figure 3 (a) Lamellipodial and filopodial extensions are filled with a network of actin filaments, organized into very specific structures. It is generally believed that the formation of these networks provides the driving force to extend the membrane. (b) An experimental model showing that actin polymerization can produce forces is provided by intracellular movement of bacteria such as *Listeria*. It has been shown that the 'comet tail' behind the bacterium has many features in common with the lamellopodium.

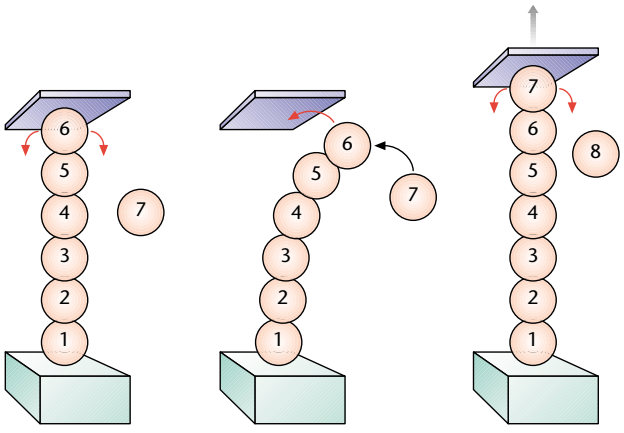

Figure 4 The production of force by polymerization raises questions about how filaments can extend when near a wall or membrane. Fluctuation-based models (Mogilner and Oster, 1996) suggest that undulations of the membrane, or of the filaments themselves, leave enough space for occasional addition of monomers. When these undulations straighten, the membrane is driven forward. Quantitative estimates indicate that, in order for this mechanism to work in the specific case of actin and cell membranes, the filaments must approach the membrane at an angle.

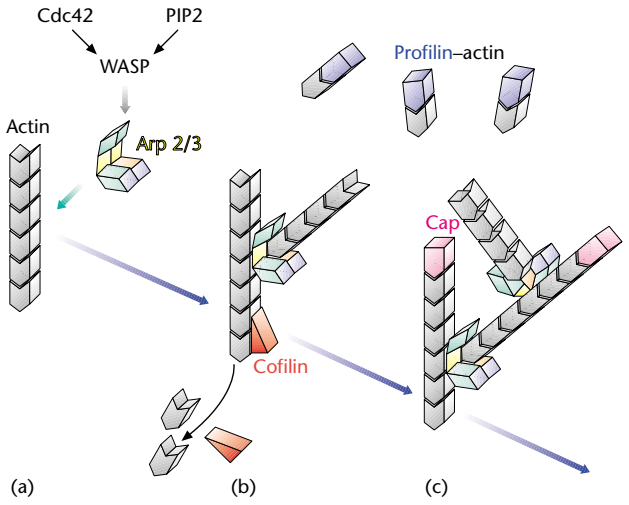

Figure 5 The formation of a branching actin network requires several accessory proteins. The key element is the Arp2/3 complex, shown in yellow-green, which is regulated by binding of a protein called N-WASP that in turn is under control of the small G protein Cdc42 and a phospholipid PIP2. The activated Arp2/3 complex binds to the sides or tips of existing actin filaments (chevron elements representing actin monomers) and nucleates the growth of new filaments at a typical angle of 70°. The source of new actin monomers is from a complex of actin with profilin, which limits extension to one end (the 'plus' end) of the polar filament. At the same time the older parts of the filaments bind cofilin, which promotes their disassembly. The extension of the filaments is also limited by binding of a capping protein to the growing ends. Each of these elements provides a means of regulating the network formation.

the soluble pool of actin monomers, and proteins that crosslink the filaments to each other or to the membrane (**Figure 5**). In the first category, the key element is a complex of seven proteins collectively called the Arp2/3 complex. Two of the main components, Arp 2 and Arp 3, are structurally similar to actin itself. Upon activation by the regulatory protein WASP the Arp2/3 complex nucleates polymerization of new actin filaments, and also can promote the growth of extensions from the sides or tips of existing ones, to which it binds. This induces formation of a dendritic network of actin with a very particular structure, having a typical branching angle of 70°. The filament network may also be driven rearward towards the cell body by this polymerization at the front. Of course, continuous assembly at the front of the cell requires a simultaneous depolymerization elsewhere in steady state. At the rear of the pseudopod, the network disassembles so as to maintain roughly constant width. This process is regulated by a protein called cofilin, which breaks up actin filaments on whose subunits the associated ATP molecule has been hydrolysed to ADP. Since the latter is a slow process relative to filament growth, cofilin preferentially degrades the older actin filaments, those likely to be found on the side of the protrusion proximal to the cell body. In addition, to keep the lengths of individual filaments short, a special capping protein blocks the growing ends. This minimal set, actin, Arp2/3, cofilin, and capping protein, are sufficient to support movement of *Listeria* bacteria *in vitro* (Loisel *et al.*, 1999).

In the cell, other components are also involved. Profilin is a protein that binds monomeric actin and facilitates its inclusion at the 'plus' (faster growing) end of the growing actin filaments. α-Actinin and other crosslinking proteins strengthen the forming actin gel, building a stronger foundation for protrusion from the front. VASP is localized at the very leading edge of the lamellipodium (Rottner *et al.*, 1999), and may serve to anchor nucleating actin filaments. VASP can also recruit profilin and so further promote actin polymerization in close proximity to the membrane. Some types of myosin are also localized at the leading edge.

A Simplified Motility System in Nematode Sperm

In different cell types the various elements of the cytoskeletal machinery are represented in different degrees, but the basic paradigm of protrusion, adhesion and retraction is maintained in all examples of crawling locomotion. The most extreme example is found in the sperm of several nematode species. Unlike other sperm types that move by beating movements of long flagella, these small cells move along surfaces very efficiently by the typical crawling mechanism. The more surprising aspect,

which makes this system principally important and instructive, is that they contain no actin. Instead, they contain a large amount of another protein called major sperm protein (MSP) whose only similarity with actin is the ability to form filaments. Otherwise, there is no sequence homology or structural similarity. Even the filaments formed from MSP are distinct from actin in that they lack a defined polarity. For this reason, it is highly unlikely that any motor protein can perform mechanical work on such filaments in a directional manner.

The common features shared by MSP and actin are more mechanistic. Both can form linear filaments by nucleation and subsequent elongation. Nucleation occurs primarily in the vicinity of the membrane. *In vitro*, bundles of MSP filaments grow from plasma membrane vesicles in a manner very similar to the formation of fibres of actin on the tails of *Listeria* bacteria, or on beads coated with the bacterial protein that promotes polymerization. In both cases the polymerization can push the nucleating objects forward. One may expect to find proteins that regulate the growth and crosslinking of the MSP filaments, though they are not yet characterized. The network disassembles at the rear of the lamellipodium, which in these cells extends all the way to the nucleus. It appears that the depolymerization of the network filaments is sufficient to induce the retraction of the cell rear. Net displacement of the cell body then follows the polymerization–depolymerization steady state in a form of treadmilling at the level of the entire network. In this case, since involvement of motor proteins is improbable, the forces responsible for retraction could originate in membrane tension that communicates the stretch at the leading edge to a forward-oriented pull at the rear. This simplified system shows that the complex phenomena required in cell locomotion are not unique to a particular material system, and therefore focuses attention on the principle of filament assembly as a driving engine.

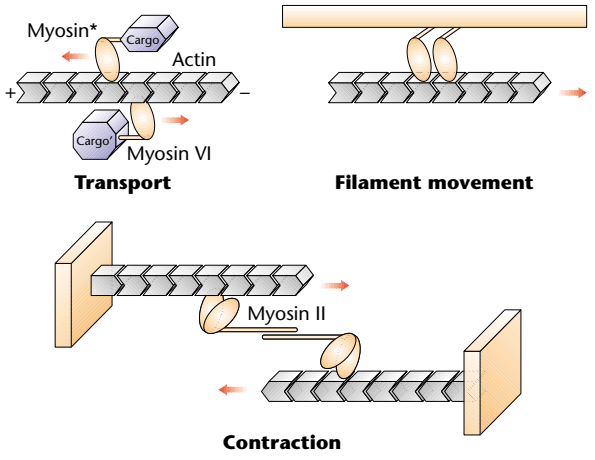

Figure 6 Many types of myosin act as molecular motors that apply mechanical forces to actin filaments. The myosins differ primarily in the tail region that governs their attachment to other cellular components. The head regions of most types (designated collectively by the asterisk) are similar in structure, and move directionally along the actin from the minus to the plus end. Myosin VI is an exception that moves in the opposite direction. This movement can drive various types of cargo along the actin. If anchored to a stationary object, myosins can also cause directional sliding of actin filaments. Myosin II has the unique ability to form bipolar filaments, which can move oppositely oriented actin arrays towards each other. It serves as the drive for most types of contraction in the cell, including muscle contraction.

The Roles of Myosins

Myosins are motor proteins, or mechanoenzymes, that convert chemical energy in ATP hydrolysis into mechanical work in moving along actin filaments. In animal cells other than the unique example of nematode sperm, movement of the cell body requires collaboration between actin and some of the myosin motors to generate the necessary pulling forces.

At least 15 different types of myosins have been described. All of them show a strong similarity in the so-called head region that interacts directly with the actin filament and displays actin-dependent ATPase activity. Myosin heads generally move in one direction, determined by the orientation of the polar actin filaments (**Figure 6**). All but one move from the slower-growing towards the faster-growing actin end (i.e. from the so-called minus to plus ends) while myosin VI moves in the opposite direction. Otherwise, the variability among myosin types is primarily in the tail portions, which have diverse functions such as cargo or membrane binding. The most familiar type is that of the muscle myosin family, the myosin IIs, where long tails of α helical rod structure can associate with one another to form dimers with oppositely oriented heads. These dimers further assemble into bipolar filaments that can organize sliding movements of oriented actin arrays into macroscopic contraction. Certain forms of myosin II also appear in nonmuscle cells. The particular myosin types responsible for cell motion remain unclear, however, as *Dictyostellium* amoeba from which myosin II was knocked out genetically are still able to move, if somewhat more slowly. In the cell types that move using fluid-driven protrusions, some actin–myosin contraction is probably required in squeezing the membrane cortex.

Polymerization itself can drive a flow of the actin network from the edge inwards within the lamellipodia. In the region behind the leading-edge protrusions, the actin network is less uniformly organized, yet actin continues to flow deep within the cell as well. This centripetal motion is a reflection of distributed contractile activity of the network, and is actually most prominent in stationary cells. This intracellular movement is presumably related to myosin activity, though it is not clear which types of myosin are involved.

In adherent cells, myosin II is involved in many aspects of the attachment between the cell and substrate, regulating the strength of adhesions, generation of forces that remodel the extracellular environment, and finally, when necessary, the disruption of cell–substrate adhesions.

Adhesion to the Substrate

Adhesion is of course required for crawling locomotion, but there is a level optimal for motility. We should therefore examine the mechanisms of cell adhesion. In higher eukaryotic cells, cells move across surfaces or within networks of proteins that form the 'extracellular matrix' (ECM). Receptors on the cell membranes interact with these proteins. In tissue cells, the integrin family is prominent among these receptors, and each matrix protein interacts with a particular set of them. The cytoplasmic parts of integrins interact with actin filaments via a complex of intermediate proteins, and thereby transmit tension from the cytoskeleton to the substrate. These adhesions are localized, rather than spread uniformly along the cell–substrate interface, and they assemble and disassemble dynamically. In highly motile cells, the main form of these so-called focal contacts is of very small clusters concentrated near the leading edge. In less motile and more strongly adherent cells, they mature into larger complexes associated with long bundles of actin and myosin II, known as stress fibres, that traverse the cell body. There appears to be a relation between the assembly of these large focal adhesions and the forces applied to the cell–substrate contacts. When development of tension is inhibited, the focal adhesions tend to disappear. There is also some feedback by which larger forces enhance the recruitment of molecular components and stimulate the assembly of focal adhesions (Balaban *et al*., 2001). Cells that grow on flexible substrates, for example, are unable to develop large tensile forces and remain without focal adhesions (Pelham and Wang, 1997). Interestingly, their speed of locomotion is higher than that of similar cells growing on a rigid support. Myosin II-dependent movement of cell–matrix adhesions can also create and form structures of extracellular matrix fibres along which the cells will then be able to move. This is especially important *in vivo* for organization of tissues.

The relationship of cell adhesion to motility goes well beyond simple sticking. The relation between tensile forces and the development of focal adhesions is one example of many phenomena known collectively as adhesion-dependent signalling, by which the cell detects both physical and biochemical properties of the extracellular matrix. Many signal transduction proteins associate with integrins in these focal adhesion complexes. These include tyrosine kinases, particularly focal adhesion kinase (FAK) and Src family kinases, as well as serine/threonine kinases. Phosphatidylinositol 3-kinase and mitogen-activated protein (MAP) kinase may be activated through these and other intermediaries. Thus integrin-mediated adhesion leads to local stimulation of protrusion, to more global cell contraction, and perhaps to long-term effects that depend on transcription. The pathway from integrin stimulation to motility-related events most probably involves regulatory GTPase proteins.

Organization of the Motility Cycle: The Role of Small GTPases

The orchestration of protrusion, adhesion and retraction into whole-cell motility is governed by a sort of biochemical computer, based mainly on a network of small GTP-binding (G) proteins (**Figure 7**). These are molecular switches that are in active form when they bind GTP and in inactive form when bound to GDP. The Rho family of small G proteins is particularly associated with regulation of the actin cytoskeleton and its functions. This family consists of several major subfamilies, including Rac, Cdc42, and Rho itself. Selective activation of Rac enhances lamellipodial activity and promotes formation of punctate focal contacts at the cell edge, while activation of Cdc42

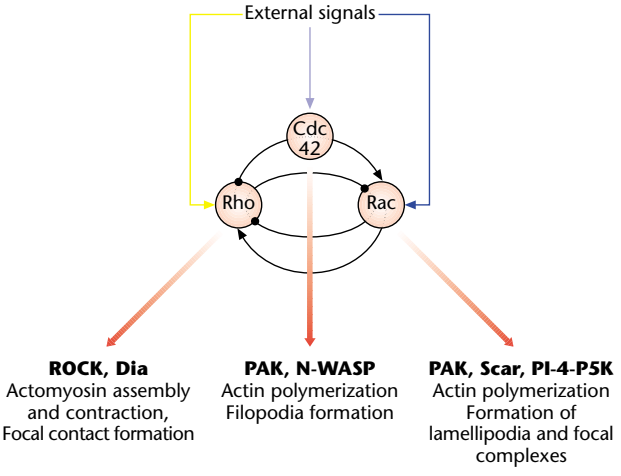

Figure 7 A network of small G proteins of the Rho family act as a sort of biochemical computer to integrate external signals and induce restructuring of the cytoskeleton. The main players are Cdc42, Rac and Rho; they are active in GTP-bound form and inactive when bound to GDP. They work via an assortment of proteins, some of which are listed in red, which lead to the reactions listed below the arrows via a further sequence of events. External factors can activate each of these G proteins via the response of membrane receptors. In addition to their effects on the cytoskeleton, several feedback paths have been identified by which these proteins regulate each other's activity. Basically, Cdc42 activates (indicated by arrowheads) Rac, and Rac activates Rho, but under some conditions Rac and Cdc42 can inhibit Rho (indicated by round head). Rho can also inhibit Rac. The interactions among these controlling elements are a subject of active research.

enhances protrusion of filopodia. Rho activation, on the other hand, leads to the development of stress fibres associated with mature focal adhesions. GTPases of other families also participate in regulation of motile activities. Arf 6 appears to work in concert with Rac in regulating lamellipodial protrusions. Each of these GTPases can activate multiple pathways, some of which are at least partly resolved. For example, Cdc42 and Rac activate protrusional activity via nucleation of actin polymerization. They do this by activating members of the WASP/Scar family of proteins, which in turn activate the Arp2/3 complex.

Rho is associated with regulation of contraction mediated by myosin II. The activity of the latter is regulated by phosphorylation of the regulatory light chain. This depends on the balance of activity of myosin light-chain kinase and myosin light-chain phosphatase. Among the targets of Rho is a kinase that inactivates the myosin light-chain phosphatase, leaving the light-chain in the active form. In addition, this Rho-associated kinase can itself act to phosphorylate the regulatory light chain. These are only a few examples of regulatory cascades. Many more exist and others remain to be discovered.

What transforms these cascades induced by small G proteins into a real regulatory computer is their ability to control and interfere with each other's activity. Activation of one, for example Cdc42, may stimulate activity of Rac, and in turn Rho, each after a certain time delay. This sequence corresponds to a phenomenology of extension of filopodia and lamellipodia, followed by changes in focal adhesions. Beside this linear order, there is evidence for back-regulation among the GTPases, particularly between Cdc42 and Rho, and Rac and Rho. This can provide the feedback necessary for oscillatory behaviour in the biochemistry to generate cyclic patterns of protrusion, adhesion and retraction.

Polarization of Cell Shape and Persistence in Directionality: Interplay between the Actin System, Adhesion and Microtubules

In addition to cycles of activity in time, motile events appear locally around the cell perimeter with different biases to the various stages. This creates a polarization in the cell shape that is required to maintain directionality in movement. The pseudopod extensions are concentrated at the leading edge in the direction of motion. The degree, and perhaps mechanism of polarization may differ among cell types. In keratocytes the protrusion and retraction are so well balanced that the cells keep a nearly constant shape as they move. Experiments using fragments of keratocytes show that disc-shaped immotile fragments can be converted to an asymmetric half-moon moving form with lamellipodia on the round side by a short mechanical stimulus (Verkhovsky *et al*., 1999). The moving form is self-sustaining, indicating that actin polymerization, depolymerization, and possibly contraction with myosin, are sufficient to maintain polarization and directionality. In fibroblasts, by contrast, local parts around the perimeter may display independent, rather asynchronous motile behaviour. The cell edge then divides into an attached, protruding and normally convex region at the leading edge, and large stable side regions, which are much more weakly attached and typically concave in profile. In the classic 'fan shape', the trailing edge is found at the junction of the concave sides. However, this picture is rather idealized and the combination of locally active and stable parts of the cell outline often produces a more complex shape.

Microtubules, the other major component of the cytoskeleton, are clearly involved in determinating polarization in large cells such as fibroblasts. Application of drugs to depolymerize microtubules leads initially to suppression of lamellipodial protrusion, followed by a reorganization of the cell shape into a more or less symmetric disc with randomly distributed quiescent and protruding regions. These nonpolarized cells are not able to migrate directionally. The primary role of microtubules is to provide a system for radial trafficking of organelles, vesicles and molecular cargo. Their function in motility may thus relate to delivery of components to sites of protrusion. In fact, the inhibition of associated molecular motors mimics the effect of microtubule depolymerization on cell shape. Microtubules also play a role in regulation of actomyosin contractility, and thereby of focal adhesions as well. Their depolymerization enhances contractility in association with an increase in the level of activated Rho and myosin II. Polymerization of microtubules is associated with an increase in the level of activated Rac and promotion of lamellipodia (Waterman-Storer *et al*., 1999). Locally, polymerization of individual microtubules can suppress the growth of focal adhesions near their ends (Kaverina *et al*., 1999). It is possible that the naturally dynamic character of microtubules provides a means to test the strength of local adhesions. Weaker ones would be eliminated, while those that survive the increased tension would be further strengthened. This positive feedback provides a mechanism for symmetry breaking and selection of a particular polarized orientation (**Figure 8**).

There are cell types, particularly the small and highly motile ones, in which microtubules are not involved in establishment of a polarized morphology. Keratocytes are perhaps the best example. Just the asymmetric distribution of myosin appears to be sufficient to maintain the polarization of these cells, or fragments of them that lack microtubules entirely. At the other extreme are cells such as neurons, whose axons are the most pronounced example of hyperstabilized edges with a protruding growth cone. This asymmetry depends on microtubules.

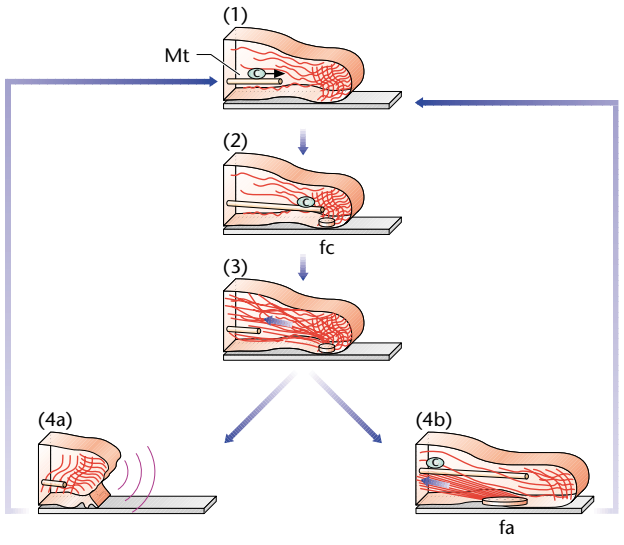

Figure 8 The scheme shows a hypothesis (Elbaum *et al.*, 1999) for the role of microtubules in development of focal adhesions and cell polarization. In (1), new actin-filled protrusions are formed ahead of the cell body. In (2), microtubules (Mt) then grow into the newly formed compartments. Microtubule growth is associated with activation of Rac (Waterman-Storer et al., 1999) and Rac stimulates the formation of lamellipodia and small focal complexes (fc). It is possible that microtubules may deliver some components (c) involved in development of these structures. The maturation and strengthening of the initial cell–substrate contacts is blocked as long as microtubules remain nearby (Kaverina *et al.*, 1999). Microtubules are dynamic structures, however, and depolymerize in a process known as dynamic instability. Depolymerization of microtubules is associated with activation of Rho (Ren *et al.*, 1999), which in turn promotes actomyosin contraction and tensile force applied to the focal complexes, as seen in (3). The contraction may cause a retraction of the cell edge (4a), or conversely the strengthening of the initial focal complexes into mature focal adhesions (fa) associated with actin bundles called stress fibres (4b). In either case the process can then repeat from step (1). In this way the dynamic instability of microtubules can test the strength of initial contacts around the cell edge, and select the strongest for further development. Such a positive feedback mechanism can lead to polarization of the shape and selection of a direction for locomotion.

External Regulation of Cell Locomotion

The role of cell locomotion in embryonic development makes clear that motility is regulated by signals of extracellular origin. These may be spatial gradients of soluble factors (classic chemotaxis), gradients of extracellular matrix proteins, contact guidance by geometric features, and adhesion contacts with neighbouring cells, all of which affect the spatial orientation of cell locomotion. Other signals may act temporally, activating or deactivating the locomotory apparatus without necessarily determining directionality. For soluble signals, the molecular pathway has been clarified in a number of cases. The general paradigm is that the presence and concentration of these signals is detected by specific transmembrane receptors. Their external stimulation leads to local polymerization of actin and development of protrusions via a number of intermediate steps, in particular the phosphorylation of inositol membrane lipids on the cytoplasmic face of the plasma membrane. The key player is the enzyme phosphatidylinositol 3-kinase (PI3-kinase), which can be activated by a number of transmembrane receptors. The resulting modified lipids then provide docking sites for a category of proteins containing a structural motif known as the pleckstrin homology, or PH domain (Haugh *et al.*, 2000). Among these are probably several that can induce the polymerization of actin. One candidate is the protein Vav2 (Pandey *et al.*, 2000) that is known to activate both Rac and Cdc42, which in turn can affect actin polymerization and formation of protrusions as described above. Certainly other regulatory proteins recognizing PI3 kinase-modified lipids are involved in regulation of actin as well. The establishment of the gradient of docking sites, inside the cell but aligned with the external gradient, operates independently of the subsequent changes in the cytoskeleton. This is one relatively well-resolved example. It is most likely that the same principle operates for other receptors detecting other types of external signals, such as those from the extracellular matrix and from adhesions with neighbouring cells. Local activation of transmembrane receptors leads to some intermediate response that establishes a gradient across the cell membrane, and finally to local modulation of small GTPases. Thus small GTPases are not only involved in the spontaneous activities relating to protrusion and contraction, but may integrate external signals and translate them into local regulation of actin-mediated locomotory events.

Concluding Remarks

We conclude with a comparison of what is currently known and what remains to be discovered. Actin polymerization is clearly a key effect in cell locomotion, but the nematode systems shows us that actin itself is not essential and that much simpler systems can achieve similar results. What, then, makes the actin system so nearly universal? Myosin is also necessary in conjunction with actin, but the distribution of functions among the various forms of myosin related to locomotion still remains to be resolved. Adhesion to an extracellular matrix via the integrin family of transmembrane receptors is widespread and essential in many motile cell types; however, the interplay between adhesion-dependent signalling and regulation of locomotion is poorly understood. The small GTPases play a central role in many aspects of this regulation. How they interact among themselves as an integrating circuit for regulation is a key question. Finally, the molecular pathways linking external stimulation of membrane receptors with modulation of the cytoskeleton are only now being revealed. How universal these pathways and

paradigms are remains a topic of current and future research.

References

Balaban N, Schwarz U, Riveline D *et al*. (2001) Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nature Cell Biology* **3**: 466-472.

Bretscher MS and Aguado-Velasco C (1998) Membrane traffic during cell locomotion. *Current Opinion in Cell Biology* **10**: 537–541.

Elbaum M, Chausovsky A, Levy ET, Shtutman M and Bershadsky AD (1999) Microtubule involvement in regulating cell contractility and adhesion-dependent signalling: a possible mechanism for polarization of cell motility. *Biochemical Society Symposia* **65**: 147–172.

Haugh JM, Codazzi F, Teruel M and Meyer T (2000) Spatial sensing in fibroblasts mediated by 3′ phosphoinositides. *Journal of Cell Biology* **151**: 1269–1280.

Kaverina I, Krylyshkina O and Small JV (1999) Microtubule targeting of substrate contacts promotes their relaxation and dissociation. *Journal of Cell Biology* **146**: 1033–1044.

Loisel TP, Boujemaa R, Pantaloni D and Carlier MF (1999) Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins. *Nature* **401**: 613–616.

Mogilner A and Oster G (1996) Cell motility driven by actin polymerization. *Biophysical Journal* **71**: 3030–3045.

Pandey A, Podtelejnikov AV, Blagoev B *et al*. (2000) Analysis of receptor signaling pathways by mass spectrometry: identification of vav-2 as a substrate of the epidermal and platelet-derived growth factor receptors. *Proceedings of the National Academy of Sciences of the USA* **97**: 179–184.

Pelham RJ Jr and Wang Y (1997) Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proceedings of the National Academy of Sciences of the USA* **94**: 13661–13665.

Ren XD, Kiosses WB and Schwartz MA (1999) Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO Journal* **18**: 578–585.

Rottner K, Behrendt B, Small JV and Wehland J (1999) VASP dynamics during lamellipodia protrusion. *Nature Cell Biology* **1**: 321–322.

Verkhovsky AB, Svitkina TM and Borisy GG (1999) Self-polarization and directional motility of cytoplasm. *Current Biology* **9**: 11–20.

Waterman-Storer CM, Worthylake RA, Liu BP, Burridge K and Salmon ED (1999) Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. *Nature Cell Biology* **1**: 45–50.

Further Reading

Bershadsky A and Geiger B (1999) Cytoskeleton-associated anchor and signal transduction proteins. Introduction. In: Kreis T and Vale R (eds) *Guidebook to the Extracellular Matrix, Anchor, and Adhesion Proteins*, pp. 3–11. Oxford: Oxford University Press. [Adhesion to the substrate]

Bishop AL and Hall A (2000) Rho GTPases and their effector proteins. *Biochemical Journal* **348**(Pt 2): 241–255. [Small GTPases]

Borisy GG and Svitkina TM (2000) Actin machinery: pushing the envelope. *Current Opinion in Cell Biology* **12**: 104–112. [Formation of actin-filled protrusions]

Bray D (2000) *Cell Movements*. London: Garland. [General review]

Chen H, Bernstein BW and Bamburg JR (2000) Regulating actin-filament dynamics in vivo. *Trends in Biochemical Sciences* **25**: 19–23. [Formation of actin-filled protrusions]

Cramer LP (1999) Organization and polarity of actin filament networks in cells: implications for the mechanism of myosin-based cell motility. *Biochemical Society Symposia* **65**: 173–205. [Myosins]

Critchley DR (2000) Focal adhesions – the cytoskeletal connection. *Current Opinion in Cell Biology* **12**: 133–139. [Adhesion to the substrate]

Donaldson JG and Jackson CL (2000) Regulators and effectors of the ARF GTPases. *Current Opinion in Cell Biology* **12**: 475–482. [Small GTPases]

Elbaum M, Chausovsky A, Levy ET, Shtutman M and Bershadsky AD (1999) Microtubule involvement in regulating cell contractility and adhesion- dependent signalling: a possible mechanism for polarization of cell motility. *Biochemical Society Symposia* **65**: 147–172. [Polarization of locomotion: interplay between actin, microtubules and adhesion]

Firtel RA and Chung CY (2000) The molecular genetics of chemotaxis: sensing and responding to chemoattractant gradients. *Bioessays* **22**: 603–615. [Regulation of locomotion by external signals]

Italiano JE, Stewart M and Roberts TM (2001) How the assembly dynamics of the nematode major sperm protein generate amoeboid cell motility. *International Review of Cytology* **202**: 1–34. [Motility of nematode sperm]

Lauffenburger DA and Horwitz AF (1996) Cell migration: a physically integrated molecular process. *Cell* **84**: 359–369. [General review]

Mahadevan L and Matsudaira P (2000) Motility powered by supramolecular springs and ratchets. *Science* **288**: 95–100. [Formation of actin-filled protrusions]

Pollard TD, Blanchoin L and Mullins RD (2000) Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annual Review of Biophysics and Biomolecular Structure* **29**: 545–576. [Formation of actin-filled protrusions]

Roberts TM and Stewart M (2000) Acting like actin. The dynamics of the nematode major sperm protein (MSP) cytoskeleton indicate a push-pull mechanism for amoeboid cell motility. *Journal of Cell Biology* **149**: 7–12. [Motility of nematode sperm]

Sastry SK and Burridge K (2000) Focal adhesions: a nexus for intracellular signaling and cytoskeletal dynamics. *Experimental Cell Research* **261**: 25–36. [Adhesion to the substrate]

Schmitz AA, Govek EE, Bottner B and Van Aelst L (2000) Rho GTPases: signaling, migration, and invasion. *Experimental Cell Research* **261**: 1–12. [Small GTPases]

Schwartz MA and Shattil SJ (2000) Signaling networks linking integrins and rho family GTPases. *Trends in Biochemical Sciences* **25**: 388–391. [Small GTPases]

Sellers JR (2000) Myosins: a diverse superfamily. *Biochimica et Biophysica Acta* **1496**: 3–22. [Myosins]

Sheetz MP, Felsenfeld D, Galbraith CG and Choquet D (1999) Cell migration as a five-step cycle. *Biochemical Society Symposia* **65**: 233–243. [General review]

Small JV, Kaverina I, Krylyshkina O and Rottner K (1999) Cytoskeleton cross-talk during cell motility. *FEBS Letters* **452**: 96–99. [Polarization of locomotion: interplay between actin, microtubules and adhesion]

Verkhovsky AB, Svitkina TM and Borisy GG (1999) Network contraction model for cell translocation and retrograde flow. *Biochemical Society Symposia* **65**: 207–222. [Myosins]

Waterman-Storer CM and Salmon E (1999) Positive feedback interactions between microtubule and actin dynamics during cell motility. *Current Opinion in Cell Biology* **11**: 61–67. [Polarization of locomotion: interplay between actin, microtubules and adhesion]

Wu X, Jung G and Hammer JA (2000) Functions of unconventional myosins. *Current Opinion in Cell Biology* **12**: 42–51. [Myosins]