

Actin dynamics *in vivo*

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Actin dynamics in lamellipodia are driven by continuous cycles of actin polymerization, retrograde flow, and depolymerization. In the past year, advances have been made in identifying signaling pathways that regulate actin-filament uncapping and polymerization, in determining the role of myosin motor proteins in retrograde flow, and in evaluating the role of severing proteins in actin depolymerization. Both *Listeria monocytogenes* and *Saccharomyces cerevisiae* have emerged as powerful model organisms for studying actin dynamics in cells.

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Abbreviations

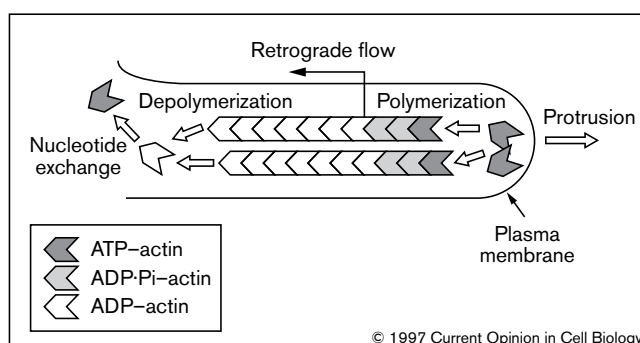
ADF	actin-depolymerizing factor
Arp	actin-related protein
BDM	butane dione monoxime
CALI	chromophore-assisted laser inactivation

Introduction

The leading edge of a motile cell is composed of thin protrusions of membrane which continuously extend and retract, mediating the initial stage of cell movement and determining the direction of advance. The underlying cytoskeleton of a leading edge is composed of actin-filament bundles (in filopodia) or meshworks (in lamellipodia) oriented primarily with their 'barbed' (fast-growing) ends towards the membrane (Fig. 1). In all cell types studied so far, leading-edge actin filaments undergo a continuous cycle of assembly at the inner-membrane surface, transport away from the membrane in a process termed 'centripetal transport' or 'actin retrograde flux or flow', and, finally, disassembly or depolymerization [1].

Here, we review recent advances in our understanding of leading-edge actin dynamics. We will address the regulation of actin polymerization, transport and depolymerization in the leading edge, and how these processes relate to the generation of motile force. We will highlight outstanding questions in the field, and emerging systems for studying these problems. We have restricted our discussion to leading-edge dynamics because this area has seen most progress recently. Actin dynamics in the cell body are less well understood, though they may be equally

Figure 1



Actin dynamics in the leading edge of motile cells. Leading-edge actin filaments undergo continuous polymerization onto filament barbed (fast-growing) ends (shown at right) at the inner surface of the plasma membrane. Newly polymerized filaments are transported towards the cell interior (towards the left) by a process called retrograde flow. Finally, filaments are depolymerized and subunits are recycled (by exchanging ADP for ATP) for a new round of polymerization.

or more important for understanding cell locomotion as a whole [2].

Actin polymerization

It has been known for some time that actin monomers are rapidly incorporated into filaments at the leading edge of cells, and it is widely accepted that polymerization of new filaments and membrane protrusion are tightly coupled. Outstanding questions include the role of polymerization in generating force for protrusion, and how polymerization is regulated. The issue of regulation has seen considerable progress over the past year, in part because it ties in to signal transduction through pathways that also regulate cell proliferation (see Tapon and Hall, this issue, pp 86–92).

The fuel for actin polymerization at the leading edge is the large cellular pool of unpolymerized actin, which is maintained at concentrations well above those needed for actin polymerization. To maintain this pool, cells employ monomer-sequestering proteins (i.e. profilin and thymosin β 4) which control polymerization through monomer availability [3] and barbed end capping proteins (i.e. CapZ and gelsolin) which regulate polymerization onto pre-existing filament ends [4,5]. The relative importance of these mechanisms may vary between cells. For example, genetic evidence suggests that profilin promotes actin polymerization in some cells and inhibits it in others [3]. Similarly, CapZ is required to prevent filament elongation in *Dictyostelium* where the concentration of unpolymerized actin is high [6••] and to protect filaments

from depolymerization in yeast where the concentration of unpolymerized actin is low [7•].

To understand how polymerization at the leading edge is controlled, we need to know the relative roles of elongation of pre-existing filament ends versus generation of new filaments by nucleation. Proteins that promote actin nucleation *in vivo* have yet to be conclusively identified. Nucleation by a templating mechanism has been proposed as a function for a complex of proteins that contains two actin-related proteins, Arp2 and Arp3 [8,9•]. This model provides an appealing analogy to the role of the γ -tubulin complex in microtubule nucleation [10,11]. Arp2 and Arp3 proteins are localized to leading-edge structures in which we might expect nucleation to be important: to filopodia and the cell cortex in *Acanthamoeba castellanii* [8,9•] and to the actively moving cortical actin patches in *Saccharomyces cerevisiae* [12•] and *Schizosaccharomyces pombe* [13•,14•]. Furthermore, mutations in the genes encoding Arp2 and Arp3 cause defects in actin organization in both yeasts [12•–14•]. These findings are consistent with a role for these Arps in nucleation, but do not rule out other functions. Study of actin-nucleation factors may be an important emerging direction.

More progress has been made both in elucidating the role of uncapping of pre-existing filaments in actin polymerization, and in identifying the signaling pathways that regulate this process. Platelets provide a useful model for studying the response of the leading edge to signal transduction pathways because they undergo a dramatic burst of actin polymerization and corresponding cell-shape change in response to various agents generated by tissue injury. This shape change is thought to be driven by actin polymerization onto barbed ends that are exposed by filament severing and uncapping [15,16], though a role for new filament nucleation has not been excluded. Recently, a new permeabilized-cell system was used to show that filament uncapping in response to thrombin-receptor activation is mediated by small GTP-binding proteins from the Rac family which work to cause an increase in the cellular levels of polyphosphoinositides [17••]. The polyphosphoinositides bind directly to the heterodimeric capping protein CapZ and dissociate it from filament barbed ends, allowing filament elongation to occur [18•]. A similar pathway involving GTP-binding proteins may also lead to filament uncapping in neutrophils [19], suggesting that this signaling pathway may be universal.

Genetic analysis of CapZ function presents a more complex picture of its role in regulating actin polymerization. In *Dictyostelium*, overexpression of CapZ causes an increase in the rate of cell motility whereas underexpression results in decreased motility rates [6••]. Similarly, overexpression of CapG (a gelsolin-related capping protein) in fibroblasts leads to an increase in motility rate and in membrane ruffling [20]. These results are difficult to reconcile with

the filament-uncapping model in its simplest form, and suggest important roles for other pathways.

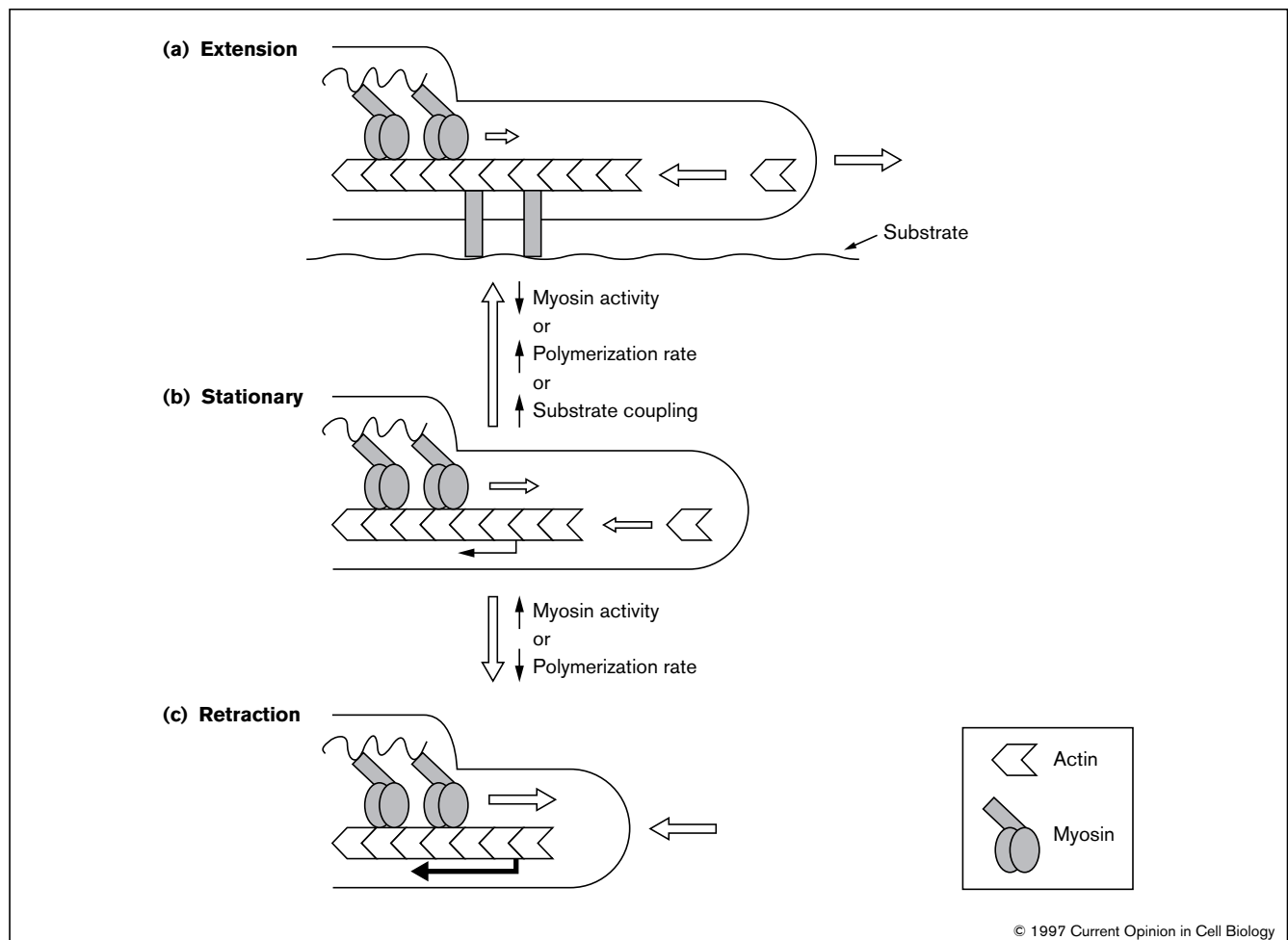
Retrograde flow

Retrograde flow of actin filaments away from the leading edge seems to be a ubiquitous feature of leading-edge dynamics. Theoretically, retrograde flow could be driven either by the free energy of actin polymerization or by ATPase motor activity. In *Aplysia* bag cell growth cones, however, retrograde flow continues at normal rates when actin polymerization has been blocked by cytochalasin treatment, demonstrating that polymerization does not drive flow [21]. In the same cell type, myosin inhibitors (e.g. the N-ethylmaleimide [NEM]-treated myosin S1 fragment and butane dione monoxime [BDM]) inhibit retrograde flow and stimulate protrusion of the cell edge [22••]. This protrusion was blocked by cytochalasin. These results suggest a simple model in which actin polymerization drives protrusion, myosin activity drives retrograde flow, and the two processes antagonize each other (Fig. 2). In this model, conditions that inhibit retrograde flow or promote polymerization lead to net protrusion, whereas conditions that promote retrograde flow or inhibit polymerization lead to retraction.

In chick dorsal root ganglion (DRG) growth cones, specific disruption of myosin I β using chromophore-assisted laser inactivation (CALI) resulted in lamellipodial protrusion [23••]. This result is consistent with myosin I β driving retrograde flow in the model, although it does not rule out the activity of other myosins. In contrast, disruption of myosin V using CALI caused a twofold decrease in filopodial protrusion rates, suggesting that this myosin promotes protrusion of filopodia. Myosin V could promote protrusion by actively driving the membrane tip forwards, in contrast with the simple model in Figure 2 but consistent with the idea that ATPase motors are required to power protrusion [24]. Alternatively, myosin V could act as the substratum-coupling factor shown in Figure 2 that prevents retrograde flow, or it could play some more complex role. Currently, the question of whether actin polymerization alone can drive protrusion remains controversial. To resolve this issue, it will be necessary to inhibit specific myosins in diverse cell types and determine the effects on both protrusion and actin dynamics.

The role of retrograde flow in cell locomotion is complex. At the leading edge, retrograde flow antagonizes protrusion. If the moving actin attaches to the substrate, however, the force that caused flow would now tend to pull the cell forwards (Fig. 2). Such attachment is likely to be regulated by signaling systems, but its molecular basis is poorly understood. In *Aplysia* growth cones, moving actin attaches to the substrate when a growth cone makes an adhesive interaction with another growth cone [25]. In *Dictyostelium*, the membrane protein

Figure 2



Model for the roles of actin polymerization and myosin activity in leading-edge motility, showing **(a)** extending, **(b)** stationary, and **(c)** retracting leading edges. Retrograde flow (shown by solid horizontal arrows pointing towards the left) results from myosin activity pulling actin filaments towards the cell interior (left). (b) At steady state, actin-filament polymerization and retrograde flow (represented by a thin solid horizontal arrow) are exactly balanced. (c) If the myosin activity is increased, thus resulting in increased retrograde flow (represented by a thick solid horizontal arrow), or the polymerization rate is decreased, net retraction will result. (a) If the myosin activity is decreased or polymerization rate increased, net protrusion will result. This effect of decreased myosin activity was seen by inhibition with BDM [22**]. Net protrusion will also result if the moving actin becomes coupled to the substrate, so that retrograde flow is inhibited. Under these circumstances the myosins will move forwards. If these myosins are tethered (represented by wavy lines at the top of the myosins) to the cell body, then this will result in a force pulling the rest of the cell forward. This effect was seen when one growth cone moved over the surface of another [25]. The identity of the myosin(s) involved in retrograde flow and the structures they are attached to are currently unknown.

ponticulins are responsible for most of the high-affinity binding between the actin cytoskeleton and the membrane [26]. Genetic ablation of this protein results in impaired chemotactic activity [27]. Three-dimensional time-lapse analysis of pseudopods (leading-edge structures in *Dictyostelium*) showed that a greater proportion of the pseudopods extended above (out of contact with) the substrate in mutant cells compared with in wild-type cells [27]. Pseudopods in ponticulins⁻ cells exhibit increased slippage and resorption into the cell body [27]. These results argue for a role for ponticulins in stabilizing the interaction of the leading-edge actin cytoskeleton with the substrate, and thus perhaps in coupling retrograde flow to forward cell movement. Whether or not ponticulins

homologs or other molecules (e.g. integrins, cadherins and myosins) play this role in mammalian cells remains to be determined.

Actin depolymerization

To maintain continuous polymerization in leading edges, actin must be depolymerized and the subunits recycled. The mechanism and regulation of actin depolymerization in cells have received less attention than the mechanism and regulation of polymerization, but there are clues from recent work that this aspect of actin dynamics is both mechanistically interesting and subject to regulation as part of the cellular response to chemoattractants and other signaling molecules.

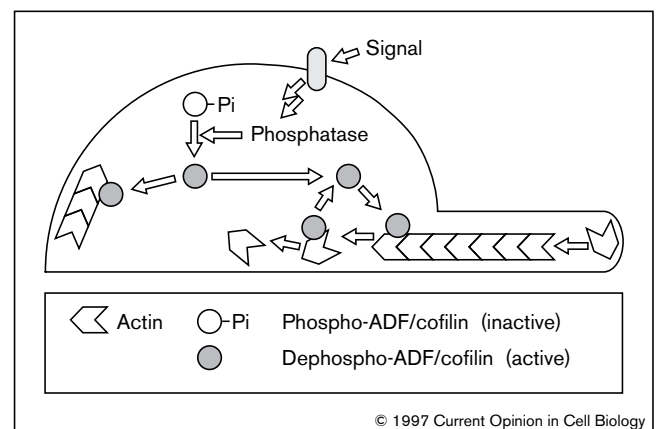
Pure actin filaments depolymerize by simple dissociation of monomers from free filament ends. At steady state they turn over by a treadmilling reaction in which subunits preferentially dissociate from pointed (slow-growing) ends and associate with barbed ends [28]. The energy for treadmilling comes from ATP hydrolysis. Pure actin treadmilling is a slow process, whose rate is limited by the low off-rate (removal of actin subunits) at pointed ends. Actin filaments in leading edges [29] and *Listeria* tails [30,31] turn over much more rapidly than would be expected from pure actin treadmilling rates, suggesting the presence of factors that either promote depolymerization from pointed ends, or else provide different turnover pathways such as filament severing. Progress in understanding the mechanism of filament turnover has been hindered by difficulties in imaging individual actin filaments at either the light- or electron-microscopic level. Recent electron-microscopic observations in keratocyte lamellipodia showed that many actin filaments are longer than previously thought [32]. This was interpreted as favoring an accelerated treadmilling mechanism for filament turnover.

Two known classes of proteins are candidates for promoting rapid actin turnover in cells: the gelsolin family, comprising highly active severing proteins that can also cap barbed ends [33]; and the ADF (actin-depolymerizing factor)/cofilin family, comprising weaker severing proteins that also possess monomeric actin-binding activity [34••]. Of course, undiscovered factors could be as, or more, important. Possible cellular functions of the severing proteins have been addressed by recent genetic approaches. Knocking out gelsolin in *Dictyostelium* produced no obvious phenotype [35]. In mice, this knockout generated a phenotype in which fibroblasts and leukocytes had slightly lowered motility rates and platelets had decreased actin-based shape changes in response to activation [36•]. Either gelsolin plays a relatively subtle role in regulating actin dynamics, or else other gelsolin-related proteins may compensate in the knockouts. In contrast, knocking out ADF/cofilin is lethal in all the organisms in which it has been tested [34••,37••,38]. This lethality impedes characterization of the effect of ADF/cofilin depletion on leading-edge dynamics; however, recent work has provided information on other cellular roles of these proteins. Before ADF/cofilin (*twinstar*) *Drosophila* mutants die, they exhibit failures in actin-dependent processes including centrosome migration and cytokinesis [37••]. Injection of inhibitory anti-ADF/cofilin antibodies into a two-cell *Xenopus* blastomere inhibited completion of cytokinesis [39••]. Thus, ADF/cofilin proteins are required for either the formation and/or the disassembly of the contractile ring. More detailed analysis of the role of ADF/cofilin in cytokinesis and leading-edge dynamics is an important future goal.

ADF/cofilin proteins appear to play an important role in the cellular response to signaling molecules (Fig. 3).

ADF/cofilin function is regulated by phosphorylation near the amino terminus of the protein (at Ser3 in chick ADF). This phosphorylation inhibits the protein's filament-severing and monomer-binding activities [40,41]. This phosphorylation site is highly conserved—indeed, two ADF/cofilin-like proteins from rat were identified solely by their ability to be dephosphorylated in response to β -adrenergic stimulation [42]. A number of signal transduction pathways that effect changes in the cytoskeleton have been found to impinge on rapid dephosphorylation of ADF/cofilin proteins (reviewed in [34••]). Identification of the relevant ADF/cofilin phosphatases and kinases is an important future goal.

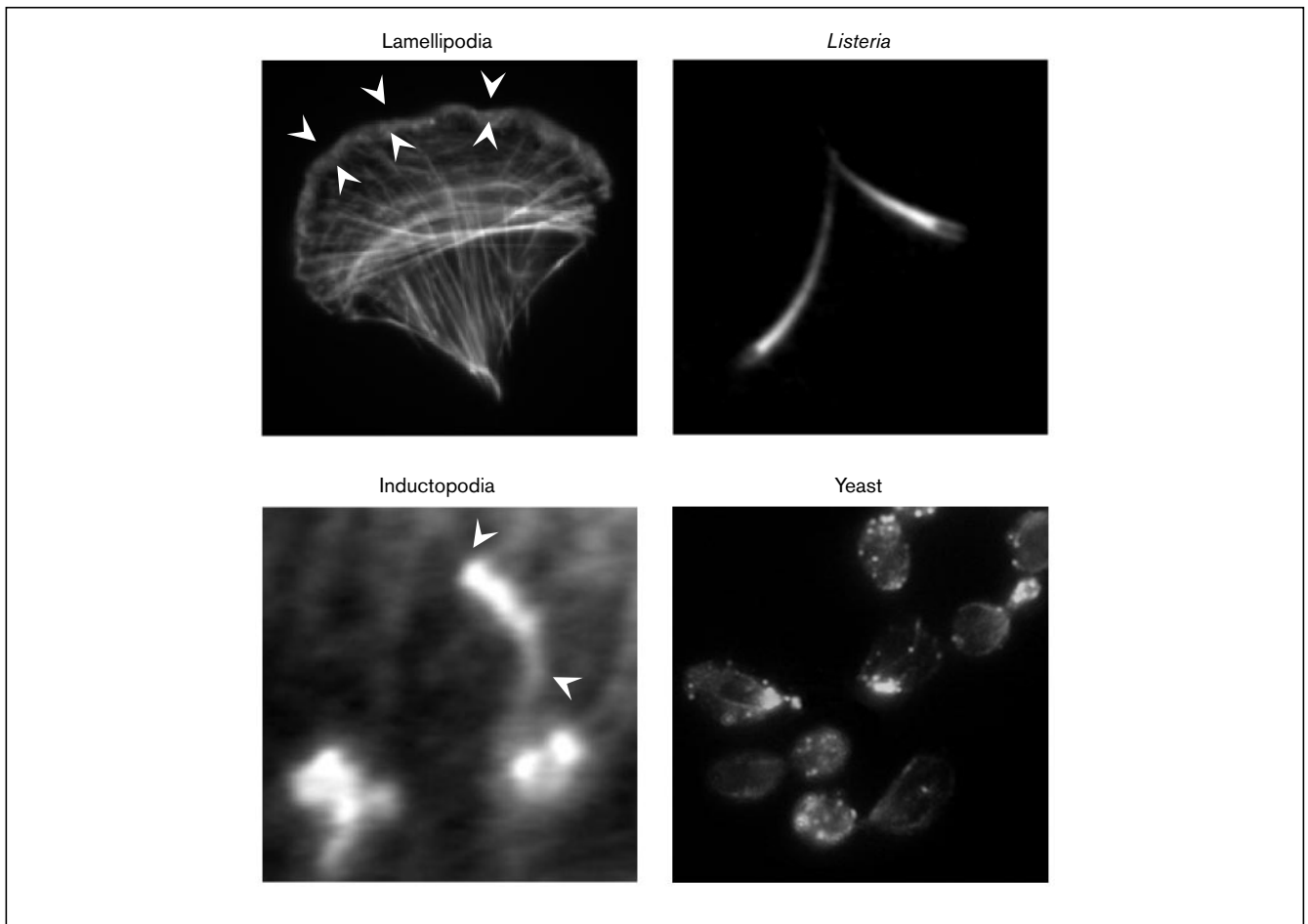
Figure 3



Activation and localization of ADF/cofilin proteins by dephosphorylation. A signal(s), such as chemoattractant, that induces cellular movement activates an unknown phosphatase which dephosphorylates ADF/cofilin. The dephosphorylated, active ADF/cofilin now translocates to regions of the cell where it promotes filament turnover. At the leading edge of the cell (protrusion at right), ADF/cofilin may promote rapid turnover, thus freeing actin monomers for new assembly. The mechanism of the ADF/cofilin-promoted turnover cycle and the role of ATP hydrolysis by actin in this cycle have not been determined.

Signaling pathways may control the temporal regulation of ADF/cofilin, but how is its activity spatially regulated? To allow cytoskeletal organization and function, actin-filament depolymerization must be restricted to appropriate locations, such as the middle and back of the leading edge. Localization of ADF/cofilin proteins correlates with their phosphorylation state, with the active, dephosphorylated form localizing to relevant areas of the cortex [39••,43•]. It is not clear whether this correlation reflects simply the greater actin-binding capability of the dephosphorylated form, or whether some more complex localization mechanism is involved. One mechanism for spatial localization is based on the preference of dephosphorylated ADF/cofilin proteins to sever ADP-actin filaments, and to spare ADP-Pi-actin (and presumably ATP-actin) filaments [34••,44]. Newly polymerized actin that has not yet released Pi after ATP hydrolysis may resist

Figure 4



Systems for analyzing leading-edge actin dynamics imaged using labeled phalloidin to stain actin filaments. The top left panel shows a locomoting fibroblast. The lamellipodium, typically $\sim 5\ \mu\text{m}$ wide, is the structure between the arrows at the leading edge of the cell. *Listeria monocytogenes* (top right panel) is a pathogenic bacterium that is pushed through cell cytoplasm by actin polymerization, leaving behind it 5–25 μm long tails (shown) composed of actin filaments. Inductopodia (see text), shown between the arrowheads in the bottom left panel, are motile foci of actin polymerization with actin tails of 5–25 μm in length that resemble those formed by *Listeria*. They are formed at the cytoplasmic face of the plasma membrane where polycationic beads rest on the extracellular surface. The cortical actin cytoskeleton of budding yeast (bottom right) is dominated by small cortical actin patches of actin (represented by the more prominently stained areas) of $\sim 0.2\ \mu\text{m}$ in diameter. Recently, these were shown to move rapidly in the plane of the cortex by a mechanism that may resemble lamellipodial protrusion and *Listeria* motility.

severing by ADF/cofilin, thus restricting severing activity to regions of the cell that are internal to polymerization sites. This mechanism might account for a restriction of depolymerization to internal regions of the leading edge. Additional mechanisms must operate in the cell as a whole, however, to allow for assembly of a complex cytoskeleton. Tropomyosin binding is thought to protect filaments from depolymerization by ADF/cofilin [45,46], but this just pushes the problem back one stage, as we do not know how tropomyosin binding is spatially regulated. Important issues for the future include addressing the spatial regulation of ADF/cofilin action, and testing whether these proteins promote actual filament severing in cells or instead promote some form of accelerated treadmilling.

Conclusions – model systems

This review has focused on recent advances in understanding actin dynamics in lamellipodia. We have highlighted progress in identifying signaling pathways that regulate actin-filament uncapping and polymerization, determining the role of myosin motor proteins in retrograde flow, and evaluating the role of severing proteins in actin depolymerization. Model systems are now being used to help to further elucidate the mechanisms that underlie actin dynamics in lamellipodia (Fig. 4). These systems present advantages in their ease of manipulation by both biochemical and genetic techniques. Two promising examples are the pathogenic bacterium *Listeria monocytogenes* and the budding yeast *Saccharomyces cerevisiae*.

***Listeria monocytogenes* and other pathogens**

Various pathogenic organisms, including bacteria (*Listeria monocytogenes*, *Shigella flexneri* and *Rickettsia rickettsii*) and viruses (vaccinia), have evolved the ability to harness actin polymerization to drive their locomotion through the cytoplasm of infected cells (for a complete treatment of this topic, see Higley and Way, this issue, pp 62–69). Of these, *Listeria* is the most studied and consequently the most well understood. *Listeria* motility is thought to be driven by actin polymerization at the bacterial surface [30,31], although roles for ATPase motor proteins have not been ruled out. Moving bacteria leave behind them a tail of actin filaments (Fig. 4) that appear to behave very much like normal leading-edge actin filaments in terms of the actin-binding proteins they recruit and their turnover rate (see Higley and Way, this issue, pp 62–69; [47,48]). Thus, it seems likely that the mechanisms by which actin filaments are polymerized and depolymerized in *Listeria* tails will be similar to those found in leading edges. *Listeria* motility can be recapitulated in cell extracts [49], and these extracts may provide the first system in which it is possible to critically test the mechanism of force generation and to identify proteins that drive actin polymerization and depolymerization. The degree to which actin dynamics in *Listeria* tails and lamellipodia are similar will probably be addressed by identifying proteins important for *Listeria* motility and by testing their role in leading edges. This will be a very active field in the next few years.

Saccharomyces cerevisiae

It might seem surprising to include yeast in a discussion of leading-edge dynamics. Yeast shares many protein components that regulate actin polymerization with higher eukaryotic cells [50] and may polarize its cytoskeleton by mechanisms related to those found in higher eukaryotes [51]. The relatively slow growth of the bud, the dominance of the rigid cell wall in morphology, and the low concentration of unpolymerized actin in the cytoplasm [7•], however, all seem inconsistent with the existence of actin dynamics like those in more conventional leading edges. Recent observations of dynamic movements of cortical actin patches (Fig. 4), made possible by green fluorescent protein tagging of actin and actin-binding proteins [52•,53•], may change this view. The cortical patches move relatively rapidly (at 0.1–0.5 $\mu\text{m sec}^{-1}$) in the plane of the cortex. Movement requires energy, but may not require the activity of known myosins [52•]. Cortical actin patches are known to be sensitive to mutations in actin and actin-binding proteins that affect actin polymerization and depolymerization [50], suggesting that polymerization dynamics play a key role in the structure and perhaps also motility of cortical actin patches. We speculate that actin patch movement may share mechanisms with such processes as leading-edge actin polymerization and *Listeria* motility. In this way, cortical patches may resemble inductopodia, which are motile foci, formed from actin, at the cytoplasmic face

of the plasma membrane where polycationic beads rest on the extracellular surface (Fig. 4) [54]. This possibility is likely to be tested in the near future by genetic analysis and also by the use of permeabilized cell systems that support actin polymerization at cortical structures [55]. Yeast actin patches may represent a better model for studying leading-edge dynamics than was previously supposed.

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