We have analysed the molecular basis of pseudopod extension using biochemical and biophysical approaches. We and others have determined the atomic structures of the key proteins including actin, profilin, Arp2/3 complex, capping protein and ADF/cofilin as well as the rate and equilibrium constants for their interactions. Arp2/3 complex interacts with actin monomers and filaments to generate new filament branches. A pool of actin bound to profilin provides subunits to elongate the ends of the branches and to push forward the plasma membrane. Capping protein terminates branch elongation. ADF/cofilin and profilin promote the disassembly of older actin filaments and the recycling of actin subunits to a pool ready for elongation of new filaments.

Students of cellular motility, marvelled for years about how cells advance their leading edges by spreading lamellae as they migrate over substrates and through the extracellular matrix. After the discovery of actin in non-muscle cells (Hatano and Oosawa, 1966), electron microscopy (Abercrombie et al., 1971; Small et al., 1978; Svitkina et al., 1997) and later fluorescence microscopy (Lazarides and Weber, 1974) revealed that actin filaments are...
concentrated in the leading lamella. Seminal experiments with photobleaching of fluorescent actin (Wang, 1985) and photoactivation of caged-fluorescent actin (Theriot and Mitchison, 1991) demonstrated that these actin filaments assemble near the leading edge and turn over on a time scale of tens of seconds deeper in the cytoplasm. More recent work with fluorescent speckle microscopy (Watanabe and Mitchison, 2002) suggests that assembly and disassembly are actually distributed processes: assembly is strongest at the leading edge but occurs deeper as well; and filaments turn over in a broad zone behind the leading edge. A key point is that external signals from chemotactic attractants and repellents guide assembly temporally and spatially. On a time scale of seconds cells can re-orientate toward a new source of attractant or turn away from repellents (Gerisch, 1982; Bourne and Weiner, 2002).

Deciphering the molecular basis of this localized assembly and disassembly of actin filaments might have proven impossible, given that it requires coordinating the activities of millions of protein molecules. However, the system has proven to be remarkably amenable to reductionist analysis coupled with insightful microscopy. All of the major components have been identified and their cellular concentrations measured in selected systems. A crystal structure of each component is available. Rate and equilibrium constants for most of the reactions are known. In vitro motility assays based on the propulsive comet tails of bacteria allow reconstitution of the whole assembly and disassembly process (Loisel et al., 1999). Many of the reactions of the purified proteins have also been visualized in real time by fluorescence microscopy (Maciver et al., 1991; Amann and Pollard, 2001; Ichetovkin et al., 2002; Fujiwara et al., 2002). Experiments in genetically tractable organisms allow tests for physiological functions, and mathematical models (Mogilner and Edelstein-Keshet, 2002) of the whole cycle of assembly and disassembly now guide mechanistic experiments.

We now have a first generation, quantitative model for the process (Figure 1.1) called the dendritic nucleation, array treadmilling hypothesis (Mullins et al., 1998a; Svitkina and Borisy, 1999; Pollard et al., 2000). This model is an over-simplification, since it considers only five protein components and ATP, but these molecules are sufficient to reconstitute motility of bacterial comet tails (Loisel et al., 1999) and thus are likely to be the core components that operate a more elaborate system in cells. I will explain our current understanding of this machine and indicate some points that need clarification or possible revision.

**Inventory of components**

*In vitro* reconstitution of bacterial motility from purified proteins (Loisel et al., 1999) showed that the essential components of the system are actin, Arp2/3...
complex, a barbed end capping protein and ADF/cofilin (Table 1.1). Profilin increases the rate of movement. An actin filament crosslinking protein, $\alpha$-actinin, also promotes movement. These are all ancient proteins with genes that arose before the earliest branches of the eukaryotic lineage. The active sites are conserved to such a remarkable degree that components from any eukaryote interact with their partners from any other eukaryote. Thus the entire system is ancient and highly conserved. It is noteworthy that genetic experiments have established that the assembly and motility of actin filament

Figure 1.1  Dendritic nucleation, array-treadmilling model for the leading edge of motile cells. (1) Chemoattractants activate plasma membrane receptors. (2) Signalling from the receptors generates activated Rho-family GTPases and PIP$_2$, which (3) activate WASp/Scar proteins on the inner surface of the plasma membrane. (4) WASp/Scar assembles a complex consisting of the Arp2/3 complex and an actin monomer on the side of an actin filament, initiating a branch. (5) Actinprofilin elongates new barbed ends, which (6) push the membrane forward until they are (7) capped. (8) Hydrolysis of ATP bound to actin subunits and dissociation of the $\gamma$-phosphate makes the filaments targets for (9) ADP/cofilin, which promotes severing and depolymerization of the filaments. (10) Profilin promotes exchange of ADP for ATP, (11) refilling the cytoplasmic pool of subunits. (12) Parallel signalling through PAK and LIM-kinase tends to stabilize filaments through phosphorylation, which inhibits ADF/cofilin. Not shown here are the events which lead to remodelling of the branched network into long, unbranched filaments. (Modified from Pollard et al., 2000). Reprinted with permission from the Annual Review of Biophysics and Biomolecular Structure, Volume 29 © 2000 by Annual Reviews
patches are defective in yeast cells lacking actin, Arp2/3 complex, ADF/cofilin or profilin.

Actin is the subunit for the filaments. Arp2/3 complex, an assembly consisting of actin related proteins Arp2 and Arp3 with five other protein subunits, initiates new filaments growing in the barbed direction as branches on the side of existing filaments (Mullins et al., 1998a). ADF/cofilin promotes the dissociation of the γ-phosphate from polymerized actin that has hydrolysed its bound ATP (Blanchoin and Pollard, 1999) and hastens the disassembly of ADP-actin filaments by severing and other effects (Bamburg et al., 1999). Heterodimeric capping protein blocks the barbed end of actin filaments (Isenberg et al., 1980). Neither budding nor fission yeast absolutely requires capping protein, but deletion of either gene encoding fission yeast capping protein is synthetically lethal with another barbed-end capping protein, so this activity is essential (D. Kovar, J. Kuhn and T.D. Pollard, submitted). In animals, gelsolin and its relatives also block barbed ends and may supplement capping protein, although cells lacking gelsolin still move (Witke et al., 1995). Profilin catalyses the exchange of nucleotide bound to actin monomers and, with capping protein, maintains a pool of unpolymerized actin far above the ‘critical concentration’ for polymerization at either end of actin filaments. Both proteins are required: profilin blocks addition of actin to pointed ends (Pollard and Cooper, 1984) and capping protein blocks the barbed ends. The actin monomer sequestering protein, thymosin-β4, is an example of a protein likely to participate in actin dynamics in animal cells (Safer and Nachmias, 1994), but which is not required for in vitro reconstitution of actin polymerization (or even found in the genomes of lower eukaryotes).

The Arp2/3 complex consists of seven subunits with structures that have been conserved since the early branches of the eukaryotic lineage (Figure 1.2;
Robinson et al., 2001; C. Beltzner and T.D. Pollard, in press, 2004). The actin related proteins Arp2 and Arp3 are folded exactly like actin, but with amino acid substitutions and longer surface loops that participate in interactions with the other subunits. ARPC1 (also called p40) is a \( \beta \)-propeller protein with seven blades similar to a trimeric G-protein \( \beta \) subunit. A novel loop inserted between blades 6 and 7 is postulated to interact with actin filaments at branches. A dimer of two similar subunits (ARPC2 and ARPC4) holds the complex together through extensive interactions of most of the other subunits. ARPC3 and ARPC5 are \( \alpha \)-helical subunits on the periphery. The conformation in the crystal is thought to be inactive, since physical separation of the two Arps prevents them from initiating a new actin filament.

The ground state of the system

In the absence of positive stimuli, physiological concentrations of these essential proteins will assemble a static gel. Roughly half of the actin will assemble into filaments and the remainder will be bound to profilin (and thymosin-\( \beta 4 \) in vertebrates). Even pure actin filaments are quite stable under physiological conditions in ATP. Owing to a small difference in the critical concentrations for elongation at the two ends (Pollard, 1986), actin subunits flux slowly onto the barbed end and off the pointed end, but the rate is less

Figure 1.2 Crystal structure of inactive bovine Arp2/3 complex, illustrating the arrangement of the seven subunits in ribbon and space filling models. Separation of the Arps is postulated to account for the inactivity of the complex. Nucleation-promoting factors are postulated to stabilize a more compact conformation with the Arps arranged like adjacent subunits in an actin filament. (Modified from Robinson et al., 2001)
than 0.1 subunit per second. Capping protein is expected to reduce treadmilling. The combination of barbed end caps and a high concentration of profilin allows cells to maintain a high concentration of unpolymerized ATP-actin ready for elongation of barbed ends when they appear.

But new barbed ends rarely appear without a positive stimulus. First, profilin inhibits the initiation of new actin filaments by spontaneous nucleation. Secondly, the Arp2/3 complex is inactive without nucleation-promoting factors. Thirdly, nucleation-promoting factors such as WASp (Wiskott–Aldrich Syndrome protein) are strongly auto-inhibited and thus inactive in the absence of positive signals. Thus the system is poised far from equilibrium ready to grow new actin filaments in response to positive stimuli.

### Signalling pathways

No signalling pathway from chemoattractants or repellents to actin is fully defined, but it is clear that multiple receptor types participate. Some are from familiar families: receptor tyrosine kinases (such as the EGF receptor), seven helix receptors coupled to trimeric G-proteins (such as Dictyostelium cAMP receptors and human leukocyte receptor for f-Met-Leu-Phe), and integrins coupled to cytoplasmic tyrosine kinases. Other receptors with yet-to-be-defined transduction mechanisms also direct actin assembly. Examples are DCC, the receptor for the growth cone attractant netrin, and Robo, the receptor for the growth cone repellent Slit (Stein and Tessier-Lavigne, 2001). Endogenous activation mechanisms independent of external stimuli are also likely to exist.

Each family of receptors has its own downstream transduction hardware, so the opportunities for complexity are immense, but several of these pathways lead to a modest number of ‘nucleation-promoting factors’ that activate the Arp2/3 complex (reviewed by Higgs and Pollard, 2001). It is important to note that alternative pathways are likely to exist: *de novo* formation of new ends by formins (Sagot *et al.*, 2002; Pruyne *et al.*, 2002) or other proteins, or multiplication of ends of existing filaments by uncapping (Glogauer *et al.*, 2000) or severing (Zebda *et al.*, 2000).

The first nucleation-promoting factor for the Arp2/3 complex to be identified was ActA from *Listeria monocytogenes* (Welch *et al.*, 1998). This transmembrane protein suffices for the bacterium to usurp the cytoplasmic actin system to assemble a comet tail. The first eukaryotic proteins shown to activate the Arp2/3 complex were the WASp/Scar family (Machesky and Insall, 1998; Machesky *et al.*, 1999; Rohatgi *et al.*, 1999; Yarar *et al.*, 1999; Winter *et al.*, 1999; Egile *et al.*, 1999). WASp is the product of the gene mutated in the X-linked immunodeficiency and bleeding disorder called Wiskott–Aldrich syndrome. Newly recognized nucleation-promoting factors
include cortactin (Weaver et al., 2001), fungal myosin-I (Evangelista et al., 2000; Lechler et al., 2000; Lee et al., 2000), fungal Abp1p (Goode et al., 2001) and fungal Pan1p (Duncan et al., 2001).

Nucleation-promoting factors activate the Arp2/3 complex, generally employing a sequence of acidic residues with a key tryptophan to bind the Arp2/3 complex. Most also bind one or two actin monomers which presumably become the first subunit(s) in the new filament. The activating part of WASp/Scar proteins is located near their C-terminus, consisting of a ‘V’ motif (for verprolin homology), a ‘C’ motif (for central or connecting) and an ‘A’ motif (for acidic). The VC region binds the actin monomer and the CA region binds Arp2/3 complex. In isolation, the VCA domain constitutively activates the Arp2/3 complex. The corresponding functional regions are located in the middle of the sequence of ActA.

Some nucleation-promoting factors are constitutively active such as ActA, but the WASp/Scar family are tightly regulated and responsive to activation by signalling pathways (Figure 1.3). WASp and N-WASp are auto-inhibited by virtue of an intramolecular interaction of the C motif

![Figure 1.3 WASp domains and activation](image)

**Figure 1.3** WASp domains and activation. Intramolecular binding of the C region to the GTPase binding domain (GBD) strongly auto-inhibits the nucleation promoting activity of the VCA domains at the C-terminus. Binding of the GBD to Cdc42 and the basic region to PIP₂ releases the VCA domains, so that the VC region can bind an actin monomer and the CA region can bind Arp₂/3 complex. Binding of this ternary complex to the side of an actin filament completes the activation process and initiates the formation of a new filament. (Redrawn from Higgs and Pollard, 2001)
with the GTPase binding domain (GBD) located in the middle of the polypeptide. This interaction prevents the VCA region from activating the Arp2/3 complex. The Rho family GTPase Cdc42 and the membrane lipid phosphatidylinositol(4,5)bisphosphate (PIP$_2$) act synergistically to overcome the auto-inhibition of WASp and N-WASp by freeing VCA to interact with the Arp2/3 complex. Other parts of WASp may contribute to activation, since the full-length protein activated by Cdc42 and PIP$_2$ is 100 times more active than VCA alone. Alternatively, the SH3 proteins Grb2 and Nck can activate N-WASp together with PIP$_2$ (Carlier et al., 2000; Rohatgi et al., 2001). The ability of multiple signalling molecules to activate WASp allows the protein to be a coincidence detector for signals flowing from diverse receptors (Prehoda et al., 2000).

Scar (also known as WAVE) lacks a GBD and is not regulated by auto-inhibition. Instead, a complex of four other proteins interferes with the ability of Scar to activate Arp2/3 complex (Eden et al., 2002). The GTPase Rac1 and the adapter protein Nck overcome this regulatory complex and allow Scar to activate the Arp2/3 complex (see also chapter by Blagg and Insall).

**Activation of the Arp2/3 complex**

Based on the crystal structure (Robinson et al., 2001) and our analysis of the activation mechanism (Marchand et al., 2001), we proposed that nucleation-promoting factors and actin filaments activate the Arp2/3 complex by stabilizing a conformation with the Arps juxtaposed like two subunits in an actin filament. We expect that the Arp2/3 complex visits this conformation rarely even without activators. Accordingly, high (micromolar) concentrations of purified Arp2/3 complex generate new actin filaments, but with a stoichiometry of only 0.001 new barbed ends per complex (Mullins et al., 1998a), reflecting the very low fraction of active complex. Therefore a small fraction of the complex must be in the active conformation at any time. Confirmation that nucleation-promoting factors stabilize an active conformation will require crystal structures or other biophysical probes of activated complexes.

Kinetic and thermodynamic analysis of the activation mechanism (Marchand et al., 2001) showed that the VCA domains from WASp and Scar bind actin and the Arp2/3 complex with submicromolar affinity and that both reactions are rapidly reversible on a subsecond time scale. This means that when WASp or Scar are activated, their VCA domains will rapidly bind the micromolar concentrations of actin and Arp2/3 complex diffusing in the cytoplasm. Given that VCA reacts faster and has higher affinity for actin than the Arp2/3 complex, we propose that when freed from
inhibition VCA first binds actin and then the Arp2/3 complex. Although the Arp2/3 complex has only micromolar affinity for the sides of actin filaments, the presence of filaments increases the affinity of the Arp2/3 complex for the VCA domain. Thus VCA binding will increase the affinity of the Arp2/3 complex for actin filaments. This thermodynamic coupling suggests that VCA and filaments favour the same active conformation of the Arp2/3 complex.

Although we had evidence that the Arp2/3 complex binds to and forms branches on the sides of existing filaments (Mullins et al., 1998a; Blanchoin et al., 2000a), Pantaloni et al. (2000) suggested that the branches actually form at the barbed end of growing filaments, with one of the Arps incorporated into the mother filament and one into the daughter filament. Direct observation of branching by total internal reflection microscopy (Amann and Pollard, 2001b; Fujiwara et al., 2002) and confocal microscopy (Ichetovkin et al., 2002) confirms that branches form on the sides of mother filaments. These real-time assays also confirmed the observation of static samples (Amann and Pollard, 2001a) that branching is favoured on newly polymerized filaments. The mechanism of this bias toward new filaments is not established, but is likely to be related to nucleotide hydrolysis and/or a structural change in the mother filament as it ages.

We do not yet know how the nucleation-promoting factors are organized in cells, but most of their activators of WASp and N-WASp are associated with membranes: PIP2 is part of the lipid bilayer; Cdc42 is tethered to the bilayer by a prenyl group; and Grb2 associates with active receptor tyrosine kinases. Thus the active fraction of WASp (and perhaps Scar) proteins is expected to be associated with the plasma membrane, making the inner surface of the plasma membrane a favoured site for activating the Arp2/3 complex. In fact, when PIP2 and active Cdc42 are incorporated into small lipid vesicles, a huge cloud of branched actin filaments grows from their surface (Higgs and Pollard, 2000). A bias toward branching from newly formed filaments would also favour nucleation near the plasma membrane where growing filaments interact with the membrane.

A key unresolved point is why branches are so much more stable than the low affinity binding of the Arp2/3 complex to the sides of actin filaments (Mullins et al., 1998b). The branch half-life in vitro is about 6 min (Blanchoin et al., 2000b) and can be prolonged by inhibiting the release of phosphate from ADP-P, subunits in the branch with phalloidin or using the stably bound phosphate analogue, BeF3. On the other hand, ADF/cofilin promotes both the dissociation of the γ-phosphate and dissociation of branches. Thus, phosphate release from subunits in the branch seems to trigger dissociation of the pointed end of the daughter filament from Arp2/3 complex. Insight into this matter is likely to require a high-resolution model of branches from cryo-electron microscopy.
Growth of the branched actin filament network

Once a branch is initiated, its free barbed end will grow at a rate \[10 \mu \text{M}^{-1} \text{s}^{-1}\] limited by diffusion of actin-profilin to the leading edge. Mogilner estimates that this reaction creates an actin-profilin sink at the leading edge, with a concentration about half that deeper in the cytoplasm (Mogilner and Edelstein-Keshet, 2002). Even so, elongation is expected to be fast, in the order of 100 subunits (0.25 \mu m) per second, enough to account for the observed expansion of the leading edge in the fastest cells. Theoretical calculations (Mogilner and Edelstein-Keshet, 2002; Mogilner and Oster, 1996; Carlsson, 2002) show that the concentration of growing filaments is sufficient to produce a force to push forward the membrane.

Electron microscopy (Svitkina et al., 1997) indicates that the branches are short, on the order of 0.5 \mu m or less, so elongation must be terminated after a few seconds. This capping rate is consistent with the rate of capping by heterodimeric capping protein estimated from its concentration and rate constant for binding barbed ends (Schafer et al., 1996).

Remarkably, most filaments in these branched networks have their barbed ends orientated toward the front of the cell. Maly and Borisy (2001) proposed a Darwinian model to explain this bias. Their model is based on the assumption that interactions of forward pointing filaments with the inside of the plasma membrane inhibits capping, whereas filaments pointing away from the membrane are capped rapidly and irreversibly. This assumption is plausible, since \( \text{PIP}_2 \) dissociates capping protein from filament ends (Schafer et al., 1996). The remarkable flatness of a typical leading lamella means that growth forward is strongly favoured relative to growth in the dorsal direction. No one has proposed a mechanism to account for this bias.

Assembly of the branched network appears to be self-organizing – an inert plastic bead coated with a nucleation-promoting factor suffices to induce a force-producing comet tail in a cell extract (Cameron et al., 1999). If the expanding network at the leading edge is anchored to the substrate via transmembrane attachments, growth of the filaments pushes the plasma membrane forward. If the actin network is not anchored to the substrate, its expansion at the leading edge results in the whole network sliding as a unit toward the cell centre. Some cells exhibit a mixture of forward motion of the cell and rear-ward motion of the network. The molecular clutch presumably consists of links between the network and cell adhesion molecules, but they still need to be identified.

Filament ageing, remodelling and disassembly

The zone of short, branched filaments at the leading edge is narrow, less than 1 \mu m wide. Actin filaments further from the front are long and unbranched
(Svitkina et al., 1997). This means that a cell extending its leading edge at 0.2 μm per second must remodel the branched network in 5 s or less. Given this brief lifetime, the branched zone will be seen only in lamellae that are actively expanding at the time of preparation for microscopy. Choosing keratocytes, cells that move rapidly at a constant rate, may have contributed to the success of Svitkina and Borisy (Svitkina et al., 1997) in preserving branches that were missing in earlier studies.

Remodelling must involve two steps: dissociation of branches; and the conversion of short filaments into long filaments. How does this happen? ATP hydrolysis and phosphate dissociation destabilize branches and are also likely to be the timer for disassembly. The rate constant for ATP hydrolysis is 0.3 s\(^{-1}\) (Blanchoin and Pollard, 2002) and nothing has yet been found that influences this reaction rate. Thus newly assembled ATP-actin subunits hydrolyse their bound ATP with a half-time of about 2 s. Phosphate dissociation is much slower, with a half-time of 350 s (Carlier, 1987; Blanchoin and Pollard, 1999), far too slow to account for debranching in cells. However, ADF/cofilin strongly accelerates phosphate dissociation from ADP-P\(_i\) actin filaments (Blanchoin and Pollard, 1999) to rates that keep pace with hydrolysis. Rate of phosphate dissociation depends on the concentration of active ADF/cofilin and involves a very low-affinity transient interaction of ADF/cofilin with the filament. Phosphorylation of ADF/cofilin by LIM kinase downstream of PAK (p21-activated kinase) (Edwards et al., 1999), blocks this and other interactions of ADF/cofilin with actin (Blanchoin et al., 2000c) and is expected to slow phosphate dissociation and to stabilize branches.

Short filaments might be converted to long filaments by subunit addition to the filament ends or by end-to-end annealing, a very favourable reaction (Andrianantoandro et al., 2001). Capping barbed ends is expected to prevent both of these reactions, so the cell must have a mechanism to avoid it. VASP appears to inhibit capping near the leading edge (Bear et al., 2002), so it may also promote annealing in the presence of capping protein.

Actin filaments in the lamella turn over on a time scale of tens of seconds. Both the pioneering photoactivation observations (Theriot and Mitchison, 1991) and recent speckle microscopy experiments (Watanabe and Mitchison, 2002) show that depolymerization occurs broadly behind the leading edge. Pure actin filaments are stable for days, treadmilling at less than 0.1 subunits per second at steady state and capping will stabilize their dynamic barbed ends. So, how do filaments turn over rapidly in cells? ADF/cofilin is the prime candidate to drive filament disassembly in cells, since ADF/cofilin and profilin increase the turnover of filaments \textit{in vitro} (Carlier et al., 1997; Rosenblatt et al., 1997). The higher affinity of ADF/cofilins for ADP-actin monomers than ADP-actin filaments provides the thermodynamic basis for their ability to depolymerize filaments (Blanchoin and Pollard, 1999) but does not reveal the
pathway of disassembly. One factor is the ability of ADF/cofilin to sever filaments, creating ends for subunit dissociation. ADF/cofilin may also promote subunit dissociation from these ends. Further work is required to firm up both the mechanism and the kinetics of disassembly.

Some cellular actin filaments, including those in stress fibres and filopodia turn over slowly. Binding of tropomyosin may contribute to their stability, since it protects filaments from severing by ADF/cofilin (Maciver et al., 1991) and also inhibits branching by the Arp2/3 complex (Blanchoin et al., 2000b).

Recycling ADP-actin subunits

ADF/cofilin binds ADP-actin tighter than ATP-actin and also inhibits exchange of the bound ADP, so it might trap ADP-actin when it dissociates from filaments. However, both profilin and ADF/cofilin are in rapid equilibria with ADP-actin (Perelroizen et al., 1994; Vinson et al., 1998; Blanchoin and Pollard, 1998). Bound profilin promotes rapid dissociation of ADP and the high cytoplasmic concentration of ATP relative to ADP results in nucleotide-free actin binding ATP (Vinson et al., 1998). ATP-actin binds profilin much better than ADF/cofilin, refilling the pool of ATP-actin (Rosenblatt et al., 1995) ready for elongation of barbed ends.

Reaction to a chemoattractant

Chemoattractants activate parallel signalling pathways employing Rho-family GTPases, which promote actin polymerization locally by at least two mechanisms: creation of new barbed ends as branches by activating Arp2/3 complex; and inhibition of ADF/cofilin, which tends to stabilize existing filaments. Alternatively, or in addition, some activators may transiently activate ADF/cofilin by dephosphorylation, promoting severing and the growth of barbed ends which are favourable for branching nucleation by the Arp2/3 complex (Zebda et al., 2000; Ichetovkin et al., 2002).

Reaction to the withdrawal of a chemoattractant

The whole system runs down automatically in the absence of a positive signal for assembly. Lacking activators, nucleation-promoting factors will return to their inhibited states. The rate of decay will be determined by the rate of GTP hydrolysis by the Rho-family GTPases. It is not known if any of the nucleation-promoting factors are GTPase activators (GAPs), like effectors of
trimeric G-proteins, but there is a large family of GAPs for the Rho-family GTPases. Without active nucleation-promoting factors creation of new filaments stops rapidly, since the Arp2/3 complex activated previously is consumed by branching nucleation. Networks in unstimulated parts of cells are predicted to disassemble in tens of seconds, perhaps hurried along by active ADF/cofilin relieved of inhibition by the PAK/LIM kinase pathway.

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