

Regulation of Actin Assembly Associated With Protrusion and Adhesion in Cell Migration

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Le Clainche C, Carrier M-F. Regulation of Actin Assembly Associated With Protrusion and Adhesion in Cell Migration. *Physiol Rev* 88: 489–513, 2008; doi:10.1152/physrev.00021.2007.—To migrate, a cell first extends protrusions such as lamellipodia and filopodia, forms adhesions, and finally retracts its tail. The actin cytoskeleton plays a major role in this process. The first part of this review (sect. II) describes the formation of the lamellipodial and filopodial actin networks. In lamellipodia, the WASP-Arp2/3 pathways generate a branched filament array. This polarized dendritic actin array is maintained in rapid treadmilling by the concerted action of ADF, profilin, and capping proteins. In filopodia, formins catalyze the processive assembly of nonbranched actin filaments. Cell matrix adhesions mechanically couple actin filaments to the substrate to convert the treadmilling into protrusion and the actomyosin contraction into traction of the cell body and retraction of the tail. The second part of this review (sect. III) focuses on the function and the regulation of major proteins (vinculin, talin, tensin, and α -actinin) that control the nucleation, the binding, and the barbed-end growth of actin filaments in adhesions.

I. INTRODUCTION

Cell migration plays a key role in many physiological and pathological processes. During embryonic development, cell migration is required for morphogenetic processes like gastrulation (98). Neural crest cells also undergo dramatic cell migration to colonize several tissues during vertebrate embryogenesis (125). The immune response is also accompanied by the movement of several cell types (129). Finally, in cancers, abnormal cell migration is a hallmark feature of metastatic cells (236).

Cell migration is a highly orchestrated multistep process. To migrate, a cell first acquires a characteristic polarized morphology in response to extracellular signals. At the cell front, actin assembly drives the extension of flat membrane protrusions called lamellipodia and finger-like protrusions called filopodia. At the leading edge of

the lamellipodium, the cell forms adhesions that connect the extracellular matrix to the actin cytoskeleton to anchor the protrusion and tract the cell body. Finally, to move forward, the cell retracts its trailing edge by combining actomyosin contractility and disassembly of adhesions at the rear (Fig. 1).

Migration varies from one cell type to another. For example, fibroblasts exhibit a relatively slow and uncoordinated movement with protruding and retracting lamellipodia, and they contain many stress fibers connected to large focal adhesions. In contrast, fish keratocytes that move faster are characterized by the presence of a unique persistent lamellipodium, small adhesions, and the absence of stress fibers and filopodia. Nevertheless, in these cells, the role and the regulation of actin dynamics associated with membrane protrusion and cell-matrix adhesion are generally accepted as common features of cell migration. In each of

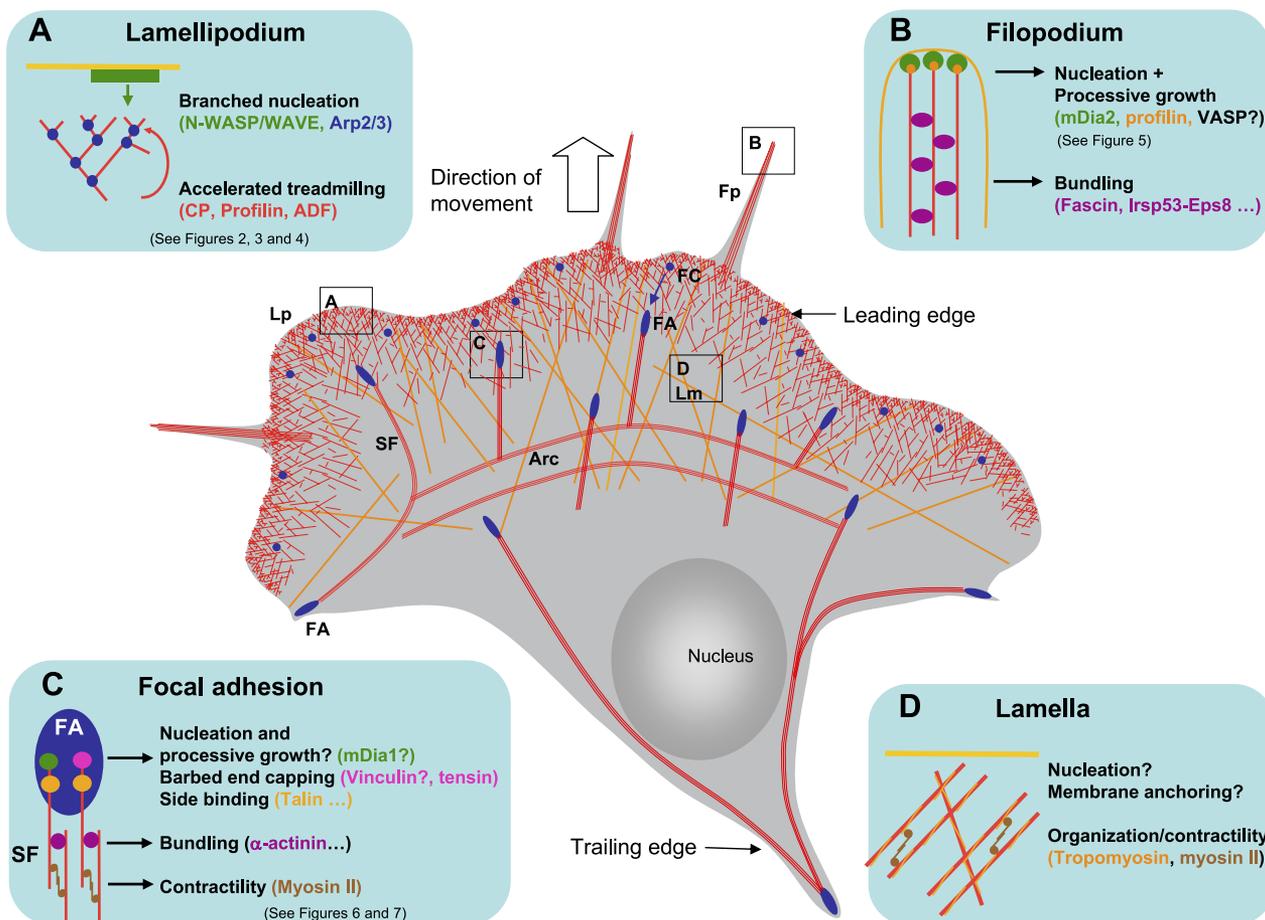


FIG. 1. Schematic illustration of the actin cytoskeleton in a migrating cell. This schematic cell contains the major structures found in migrating cells but does not correspond to a precise cell type. *A*: in lamellipodia, branched actin filaments are generated at the plasma membrane by the signal responsive WASP-Arp2/3 machinery and maintained in fast treadmilling by a set of regulatory proteins (ADF, capping proteins, profilin). The mechanism of branched nucleation is detailed in Figure 3, the regulation of WASP proteins is developed in Figure 4, and the regulation of actin treadmilling is presented in Figure 2. *B*: during cell migration, cells extend fingerlike protrusions called filopodia beyond the leading edge of protruding lamellipodia to sense the environment. At the tip of filopodia, formins like mDia2 catalyze the processive assembly of profilin-actin. The resulting nonbranched actin filaments are tightly bundled by several proteins including fascin and the Irsps53-Eps8 complex. The processive assembly of profilin-actin catalyzed by formins is presented in Figure 5. Note that VASP is also present at the tip of filopodia, but its role is still controversial (see sect. II D). *C*: slow moving cells form focal adhesions in response to RhoA signaling. Focal adhesions connect the extracellular matrix to contractile bundles made of actin filaments, myosin II, and bundling proteins including α -actinin. Focal adhesions contain a variety of actin binding proteins including side binding proteins, capping proteins, and nucleators. *D*: the lamella is characterized by a slow actin turnover and contains the signature proteins tropomyosin and myosin II. The mechanisms by which actin filaments are nucleated and anchored at the plasma membrane are not known. Lp, lamellipodium; Fp, filopodium; Lm, lamella; SF, stress fiber; FA, focal adhesion; FC, focal complex.

these cellular processes, actin dynamics are the result of a concerted regulation of parameters that govern the assembly, stability, and organization of actin filaments by a specific set of proteins.

To understand how actin dynamics control cell migration, this review addresses the following basic questions: How is rapid actin assembly maintained at steady state in the lamellipodium during cell migration? How is actin assembly constantly initiated in a site-directed fashion to account for directional migration? How is actin assembly initiated, regulated, and mechanically coupled to adhesion to enable protrusion and traction of the cell body? What are the functions of the actin binding proteins present in cell-matrix adhesions?

The first part of this review (sect. II) focuses on our current understanding of how cells extend protrusions in the direction of migration. We detail the regulation of actin treadmilling that is considered as the engine that generates the protrusive force in lamellipodia. We also describe the signal responsive machineries that finely tune the nucleation of actin filaments and direct the protrusion. The formation of different protrusions, such as lamellipodia and filopodia, in the same cellular environment, can be explained by the activation of two different nucleation machineries (Arp2/3 and formins) that generate actin arrays with different architecture and dynamics (Fig. 1).

The second part of this review (sect. III) focuses on the function and the regulation of actin dynamics at sites

of adhesion to the extracellular matrix. To convert the polarized treadmilling into protrusion and the actomyosin contraction into traction of the cell body and retraction of the tail, cell-matrix adhesions mechanically couple actin filaments to the substrate. This coupling involves a variety of actin binding proteins that control the nucleation, the binding, and the barbed-end growth of actin filaments (Fig. 1). These proteins may tune the degree of force transmission to the substrate. Although the existence and the mechanism of actin nucleation are still unclear, we describe several hypotheses that involve known actin nucleators such as Arp2/3 and the formin mDia1 in this process. Finally, we present the function and the regulation of four major actin binding proteins: vinculin, talin, tensin, and α -actinin.

II. ACTIN-BASED PROTRUSIONS

A. The Lamellipodium and the Lamella

How actin assembly is converted into a protrusive force is a central question in understanding cell migration. Seminal experiments showed how actin dynamics are coupled to the protrusion of the lamellipodium, a flat cellular protrusion where actin is organized in a bidimensional dendritic array of branched filaments (see Ref. 206 for a review) (Fig. 1). First, the microinjection of fluorescent actin followed by fluorescence recovery after photobleaching (FRAP) analyses in the lamellipodium of a fibroblast demonstrated that actin filaments polymerize at the leading edge and depolymerize at the rear in a process known as “treadmilling.” This observation suggested to the authors that this process is the result of the treadmilling of filaments whose length corresponds to the width of the lamellipodium (237). Second, the movement of a photoactivated fluorescent actin mark in the lamellipodium of a moving keratocyte remains stationary with respect to the substrate, indicating that the movement occurs at the same rate as actin assembly. In this experiment, the exponential decay of actin fluorescence suggested that actin filaments, which are nucleated and released from the plasma membrane, are short and depolymerize rapidly in the cytoplasm, ruling out the hypothesis of the treadmilling of long actin filaments (224). However, because the pointed end depolymerization of actin filaments all along the lamellipodium feeds the fast elongation of actin filament barbed ends at the leading edge, the treadmilling concept has been extended to the whole actin network. Depending on the degree of mechanical coupling between the actin cytoskeleton and the substrate, the treadmilling is mainly converted into protrusion in fast-moving cells and retrograde flow in slow-moving cells. The treadmilling is responsible for the retrograde flow at the front of the lamellipodium, whereas myosin II activity generates this flow at the back.

Until recently, it was thought that the treadmilling of actin filaments in the lamellipodium was sufficient to support the protrusion of the leading edge. However, studies based on fluorescence speckle microscopy proposed that two distinct but overlapping actin networks are involved in this process. Fast-moving and short-lived speckles defined the lamellipodium, and slow-moving and long-lived speckles defined the lamella (88, 167). The observation of the lamella behind the lamellipodium in electron microscopy showed a loose array of unbranched actin filaments (217) that is enriched in tropomyosin and myosin II (51, 219). The microinjection of skeletal muscle tropomyosin in cells displaces Arp2/3 and actin depolymerizing factor (ADF) (see sect. II, B2 and C) from the leading edge, resulting in the loss of the lamellipodia. Surprisingly, this treatment increases leading edge protrusion persistence and migration rate (73). Based on these observations, the authors suggested that the lamella plays the major role in protrusion, whereas the lamellipodium is dispensable. The lamellipodium would serve another function such as sensing the environment. Another possibility is that the lamellar and lamellipodial actin networks have redundant functions in cell migration.

B. Actin Treadmilling

1. Molecular basis of actin treadmilling

Actin assembly is a dissipative biochemical process in which actin hydrolyzes ATP upon polymerization, thus creating a difference between the critical concentration (C_c) of the ATP-bound barbed end ($C_c = 0.06 \mu\text{M}$) and the ADP-bound pointed end ($C_c = 0.6 \mu\text{M}$). At steady state, the value of the concentration of monomeric actin ($C_{ss} = 0.1 \mu\text{M}$) implies that the barbed end elongation balances the pointed end depolymerization so that on average the filament moves forward and keeps the same length. This process is called “treadmilling.” The resulting force is thought to push the membrane to form the lamellipodium. Therefore, the control of actin depolymerization at the pointed end and polymerization at the barbed end determines the protrusive force. Because the treadmilling of pure actin is too slow to account for the fast locomotion of cells, many studies have been carried out to identify actin binding proteins that accelerate the treadmilling cycle. The following paragraphs detail the molecular mechanisms by which a set of proteins (ADF, profilin, and capping proteins) cooperate to accelerate the treadmilling rate (Fig. 2).

2. ADF

ADF (also called cofilin) is localized throughout the lamellipodium but excluded from the leading edge (217). In vitro, ADF binds to the sides of the ADP-actin filament

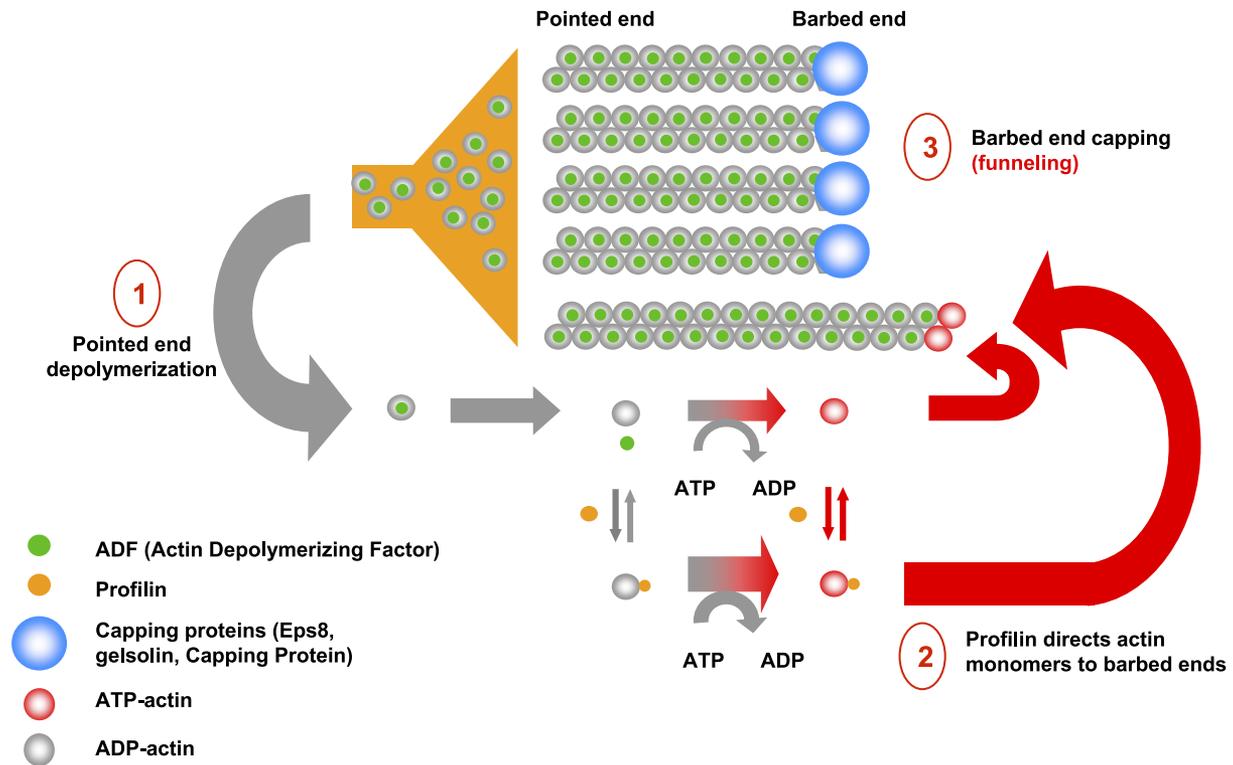


FIG. 2. Regulation of actin treadmilling. 1) ADF binds to the side of ADP-actin filaments and induces pointed-end depolymerization to increase the concentration of monomeric actin at steady state. 2) Profilin enhances the exchange of ADP for ATP to recycle actin monomers. The profilin-actin complex assembles exclusively at the barbed end. 3) By blocking the majority of actin filament barbed ends, capping proteins increase the concentration of monomeric actin at steady state and funnel the flux of actin monomers to the noncapped filaments, which individually grow faster.

and changes its structure, which causes an increase in the rate of pointed end depolymerization (30, 32). As a result, partial depolymerization occurs and the steady-state concentration of G-actin (monomeric actin) increases up to a new value that allows barbed end growth to balance faster pointed end depolymerization. Hence, it is by increasing the rate of depolymerization that ADF promotes a higher rate of barbed end growth and potentially fosters actin-based motility (Fig. 2). In addition, the change in twist of the filament linked to ADF binding (140) weakens the structure and causes a modest severing effect, resulting in a two- to threefold decrease in average length (30, 131). Based on real-time imaging of Oregon green actin filaments, it was recently proposed that, at low concentration, ADF promotes the depolymerization by severing actin filaments, not by increasing the dissociation between subunits, whereas it nucleates actin filaments at higher concentrations (6). It must be taken into account that these data were obtained exclusively with a high percentage of biochemically uncharacterized fluorescently labeled actin. We showed that labeling actin with TMR alters the functional interaction of many actin binding proteins including ADF (164). Most importantly, the severing does not explain the effect of ADF on actin-based motility. The severing in itself is not catalytic, does

not depolymerize F-actin, and does not increase the critical concentration (30) required to increase the velocity of processes driven by barbed end growth.

In good agreement with its biochemical function, ADF increases the velocity of *Listeria* and N-WASP-coated beads in reconstituted motility assays and decreases the length of the actin tails (30, 249). Identical effects are reported in vivo, where the RNAi knockdown of ADF in *Drosophila* S2 cells increases the width of the lamellipodial actin network (88).

The activity of ADF is regulated by phosphorylation. The LIM kinase phosphorylates ADF and inactivates it, whereas the phosphatase slingshot activates it (8, 151).

3. Profilin and WH2-containing proteins

Profilin is an abundant protein that binds monomeric actin and also contributes to increase the treadmilling rate (see Ref. 258 for a review). The profilin-actin complex assembles exclusively at the barbed end; thus it enhances the directionality of treadmilling (Fig. 2). The combined effects of ADF and profilin enhance the treadmilling rate by 125-fold (52).

The WH2 (WASP homology 2) domain proteins include functional homologs of profilin. The WH2 domains

are found in a variety of proteins divided into four major subfamilies: proteins with WH2 repeats like actobindin and ciboulot that promote barbed end assembly in a profilin-like fashion (20, 79), proteins that sequester actin monomers like thymosin $\beta 4$ (161), signaling proteins like WASP in which the WH2 domain contributes to the Arp2/3-dependent mechanism of nucleation (see sect. II C5A), and proteins of the spire family that nucleate actin filaments (172). A structural and mutagenetic study showed that the weaker interaction of the COOH-terminal region of the first WH2 domain of ciboulot is responsible for the opposite function of ciboulot and thymosin $\beta 4$ (78). Recently, Co et al. (40) demonstrated the importance of the WH2 domain of N-WASP in actin-based motility. They suggested that barbed end capture by N-WASP WH2 domains is required to propel N-WASP-coated beads (see sect. II C1) (40).

4. Barbed end capping proteins

Capping proteins cap the barbed ends of actin filaments with high affinity (195). By blocking the majority of the barbed ends, capping proteins increase the concentration of monomeric actin at steady state to the critical concentration of the pointed end. This higher concentration of G-actin feeds the rare noncapped filaments that grow faster in a process known as “funneling” of treadmilling (see Ref. 162 for a review) (Fig. 2). Capping proteins also control the density of the actin array by limiting the length of actin filaments (249).

A number of capping proteins involved in different cellular functions and controlled by distinct signaling pathways have been identified. The heterodimeric capping protein (CP) controls lamellipodium protrusion (182). Both phosphatidylinositol 4,5-bisphosphate (PIP₂) and the protein CARMIL inhibit actin filament capping by CP (195, 256). Whether this uncapping mechanism contributes to the initiation of actin assembly in response to signaling is still an open issue. Proteins of the gelsolin family such as the capping and severing protein gelsolin and the capping protein CapG are involved in cell migration (see Ref. 203 for a review). However, the CapG/gelsolin double knockout mice have only mild defects, suggesting a redundancy for the capping function (250). Tensin is a large modular protein that caps actin filaments in focal adhesions (see sect. III C2c). Twinfilin combines ADP-G-actin sequestering and ADP-bound barbed ends capping to regulate actin assembly associated with endocytosis (75). Eps8, an autoinhibited capping protein activated by the signaling protein Abi1, localizes along the leading edge of lamellipodia and supports filopodia formation and efficient motility of *Listeria* in host cells (53, 54). Finally, Hip1R forms a complex with cortactin that blocks actin filament barbed end elongation associated with endocytosis (116).

The dynamics of capping protein interaction with barbed ends in vitro have been examined by modeling the kinetics of barbed end growth from spectrin-actin seeds in the presence of capping protein. The derived dissociation rate constant was very low (0.0005 s^{-1}) (195). The dynamics of capping protein interaction with barbed ends in vivo have been addressed more recently using fluorescence speckle microscopy (88, 146). Surprisingly, the dissociation constant derived from speckle lifetime appears three orders of magnitude shorter in vivo than in vitro (0.58 s^{-1}). The RNAi knock down of ADF increases the lifetime of barbed end-bound capping protein (88, 146). These data indicate that in vivo the dissociation of capping protein is linked to filament depolymerization from the pointed end, which eventually releases the capper in the cytoplasm. The average lifetime of capped filaments should then be identical to the time of residence of the capper at barbed ends, whereas speckle microscopy of filaments indicates that it is longer. To explain these unexpected results, several hypotheses have been made: an unknown factor uncaps filaments rapidly, the capped filaments are preferentially depolymerized by ADF, or the severing activity of ADF near the barbed end accelerates the depolymerization of the capped barbed ends (88, 146). The measurement of the length distribution of actin filaments in the zone of capping proteins would help to understand these observations.

C. Nucleation of Actin Filaments by the Arp2/3 Complex in Response to Signaling

1. Nucleation is required to maintain the turnover of a stationary actin array in the presence of a barbed end capper

To maintain the treadmilling rate constant, migrating cells nucleate actin filaments continuously to balance the effect of capping proteins. In the lamellipodium of migrating cells, the nucleating factor is the Arp2/3 complex activated by the signaling proteins WASP (see Ref. 70 for a review).

Listeria, *Shigella*, or N-WASP-coated beads placed in a minimum motility medium containing the pure proteins ADF, profilin, capping proteins, and Arp2/3 move at the same rate as lamellipodia protrude (126, 249). In agreement with this study, all these proteins are required for lamellipodium extension in *Drosophila* S2 cells (182). Altogether, these observations demonstrate that, in migrating cells, lamellipodium extension is a self-organized process resulting from the site-directed nucleation of a polarized actin array maintained in rapid treadmilling by a combination of soluble proteins. In addition to fuelling the treadmilling with new actin filaments, the localized activation of Arp2/3 directs the protrusion.

2. The Arp2/3 complex

The Arp2/3 complex was initially discovered in *Acanthamoeba* (130; see Refs. 70, 80 for reviews). The Arp2/3 complex localizes at the leading edge of migrating cells (217) where it nucleates branched actin filaments (see sect. II C 3). Arp2/3 is a stable complex of seven conserved subunits including the two actin-related proteins Arp2 and Arp3 and ARPC1, ARPC2, ARPC3, ARPC4, and ARPC5. In humans there are two isoforms of Arp3 (Arp3 and the Arp3 β) and ARPC1 (ARPC1A and ARPC1B) (80). Whether the incorporation of these different subunits provides different activities or regulations to the complex is still an open issue. The reconstitution of human recombinant Arp2/3 complex provided multiple insights into the role of the individual subunits in the stability of the complex and the nucleation of branched filaments. In particular, this study showed that ARPC2 and ARPC4 form the structural core of the complex, Arp3 is involved in the nucleation process, and ARPC1, ARPC3, and ARPC5 contribute to the activation of the complex by WASP and ActA (72). The crystal structure of the bovine Arp2/3 complex in its inactive state confirmed the structural similarities of Arp3 and Arp2 with actin and the central role of the core proteins ARPC2 and ARPC4. Nevertheless, major structural changes are required to account for the mechanisms of branched nucleation (179).

3. Mechanisms of branched nucleation

The comparison of actin and the two Arp subunits showed that the differences in sequence are found mainly at the pointed end while the barbed end is conserved. Based on these observations, Kelleher et al. (97) predicted a model in which a complex Arp2-Arp3-actin mimics an actin nucleus to initiate actin assembly from its barbed end. The first biochemical studies and electron microscopy observations suggested that the Arp2/3 complex was a nucleator, a pointed end capper, and a branching agent (148). Direct observation of actin filaments labeled with rhodamine-phalloidin in fluorescent microscopy suggested that Arp2/3 activated by the VCA domain of WASP proteins (see sect. II C 5A) nucleated actin filaments by branching the side of preexisting filaments (4, 16). Actin filament branches are not stable and dissociate from the mother filament with a half-time of 500–800 s (17, 115). Finally, the real-time observation of actin filament branching using total internal reflection fluorescence microscopy (TIRFM) showed that branching occurred preferentially near the barbed end (4). Based on these findings, the authors proposed the “dendritic nucleation” model in which the Arp2/3 complex binds to the side of a preexisting filament and initiates a lateral branch (Fig. 3).

In contrast, based on the following findings: the autocatalytic nucleation of actin filaments by the Arp2/3 complex depends on the number rather than the length of

actin filaments, mother filaments capped at their barbed ends do not branch, the lengths of mother and daughter filaments are nearly identical, an alternative mechanism was proposed in which the complex Arp2/3-VCA-G-actin branches actin filaments at their barbed end (21, 160) (Fig. 3). These kinetic data do not provide structural information on the organization of the different subunits of Arp2/3 and VCA-bound actin at the branch junction. In particular, they do not discriminate between a model in which one Arp subunit would incorporate into the mother filament (160) and an alternative model in which the actin monomer bound to the WH2 domain of VCA would incorporate at the barbed end of the mother filament, thus positioning Arp2-Arp3 complex to initiate the daughter branch on the side of the barbed end of the mother filament (115). A recent study that combined fluorescent labeling of Arp2/3 subunits and cryo-EM localized Arp2 and Arp3 in the daughter branch, favoring the second model. The authors also showed that the Arp2/3 complex associates with the mother filament with its long axis aligned perpendicular to the mother filament with the barbed ends of Arp2 and Arp3 facing the direction of the daughter filament growth (57).

4. ATP binding and hydrolysis on Arp2/3

ATP exchange and hydrolysis, which control the recycling of actin monomers and the stability of actin filaments, play a critical role in actin treadmilling. Sequence analysis and structural modeling predicted that the amino acids involved in ATP binding and hydrolysis on actin are conserved in both Arp2 and Arp3 (97, 136), raising the question of the role of the nucleotide bound to actin, Arp2, and Arp3 in the nucleation/stability of branched filaments.

Photo-cross-linking, gel filtration, and ϵ -ATP fluorescence measurements showed that the inactive Arp2/3 complex binds one ATP with high affinity on Arp3. The binding of N-WASP to Arp2/3 reveals a second high-affinity site on Arp2 that is required for branched nucleation (114). Mutations in the ATP binding pocket of both Arp2 and Arp3 confirmed the critical role of ATP binding to Arp2 in the branched nucleation process (69, 136). Different conditions of photo-cross-linking experiments led others to propose that the inactive Arp2/3 binds ATP on both Arp2 and Arp3 with similar affinities (48, 136). However, ATP- ϵ -ATP exchange was unaffected by mutations abolishing ATP binding to Arp2 but drastically impaired by mutations on Arp3, consistent with the view that Arp3 but not Arp2 binds ATP tightly in the absence of N-WASP (136). The resolution of the crystal structure of nucleotide free, ATP, or ADP-bound Arp2/3 indicated that the binding of ATP to Arp3 is important to maintain the structure of the cleft in its closed conformation, whereas ATP did not change the structure of Arp2 in which subdomains 1

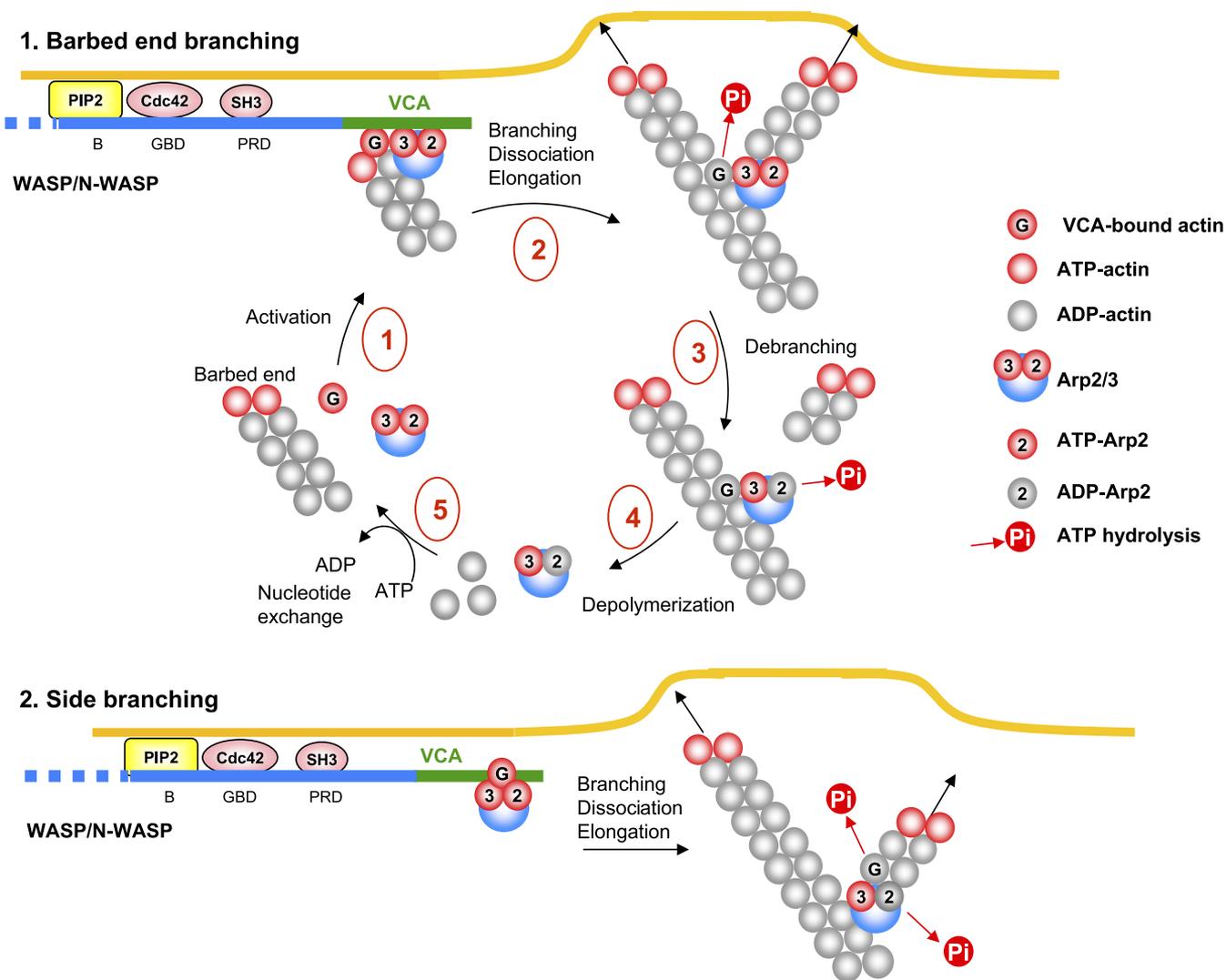


FIG. 3. Model for the catalytic cycle of branching by Arp2/3. *Top*: the barbed end branching model. 1) The branching complex made of G-actin, Arp2/3, and the VCA domain of WASP/N-WASP (or WAVE isoforms) binds to the barbed end of the mother filament. This incorporation is mediated by the actin subunit of the branching complex. 2) Arp2/3 nucleates a lateral branch. ATP hydrolysis on actin dissociates the branched junction from the membrane-bound activator. The growth of the mother and daughter filaments drives membrane protrusion. 3) ATP hydrolysis on Arp2 induces debranching. 4) After depolymerization of the branched filament, Arp2/3 and actin are released. 5) Nucleotide exchange is required to recycle actin and Arp2/3. The duplication of barbed ends at the plasma membrane can easily account for the formation of a polarized dendritic actin array in which the barbed ends face the plasma membrane. *Bottom*: the side branching model. This model proposes that Arp2/3 activated by the COOH-terminal domain of WASP/N-WASP (or WAVE isoforms) binds to the side of an actin filament. In this activated state, Arp2-Arp3-G-actin mimics an actin nucleus to initiate a lateral branch.

and 2 are flexible and absent from the electron density maps (153, 179). These observations may explain why Arp3 binds ATP with high affinity and Arp2 binds ATP with low affinity in the inactive complex (114).

ATP is not hydrolyzed on Arp2/3 alone or associated with N-WASP (114). ATP is hydrolyzed on Arp2, not on Arp3, after a delay following filament branching (115). Depending on the experimental procedure, the delay was found to be 800 s (115) or a few seconds (49, 135). These kinetic data clearly showed that ATP hydrolysis is not involved in branched nucleation but more likely in a later reaction. Branched actin filaments

carrying a nonhydrolyzable analog of ATP on Arp2/3 did not debranch, demonstrating that ATP hydrolysis on Arp2 but not on actin is a prerequisite for debranching (115) (Fig. 3). In agreement with our findings, mutations in amino acids of Arp2 required for ATP hydrolysis did not affect the branched nucleation but strongly inhibited debranching (135).

Debranching is slower than the renewal of actin filaments in the lamellipodium. Therefore, the debranching is likely to be regulated to contribute to the destabilization of actin arrays *in vivo*. Several observations support this hypothesis. First, Martin et al. (135) showed recently

that a yeast mutant blocked for ATP hydrolysis on Arp2 and debranching displayed longer actin tails associated with endocytic sites. Second, in the lamellipodium of *Drosophila* S2 cells, fluorescent Arp2/3 speckles disappear faster than actin speckles, indicating that debranching is not the result of actin depolymerization. The short lifetime of Arp2/3 speckles (22 s) also showed that debranching is much faster in vivo than in vitro ($t_{1/2} = 500\text{--}800$ s), suggesting the existence of an unknown mechanism of regulation (88). This observation could reflect the binding of ADF to the side of ADP-actin filaments that is known to accelerate the debranching rate in vitro (17). Altogether, these observations suggest that ATP hydrolysis on Arp2/3 plays the role of a timer regulating the plasticity of the branched actin array, making it softer at a distance from the leading edge. Since debranching releases free depolymerizing pointed ends, it is tempting to anticipate that the regulation of debranching not only controls the organization and the mechanical properties of the actin array but also the treadmill rate.

5. Activation of Arp2/3

The establishment of the polarity of the lamellipodial actin network is a critical event in the initiation of cell migration. Studies on the propulsion of the bacteria *Listeria* revealed that Arp2/3 is required for the formation of the polarized actin tail that propels the bacteria in the host cell. The discovery that Arp2/3 is activated by the *Listeria* transmembrane protein ActA (247, 248) strongly suggested that, in cellular processes like lamellipodium protrusion, Arp2/3 must be activated by membrane-associated signaling proteins to initiate the growth of a polarized actin network. The following paragraphs describe the signaling pathways leading to the activation of Arp2/3. Note that this part of the review does not cover the nonmammalian activators of Arp2/3 (see Ref. 70 for a complete list).

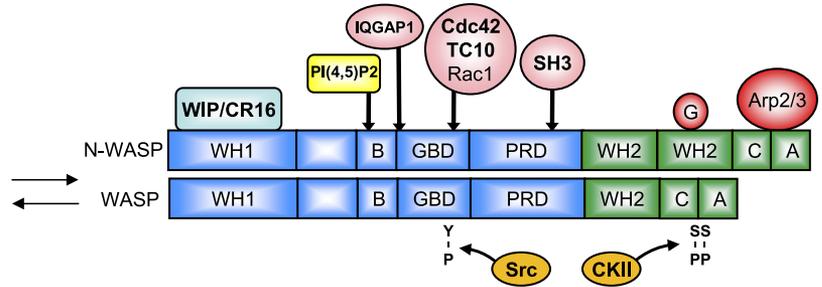
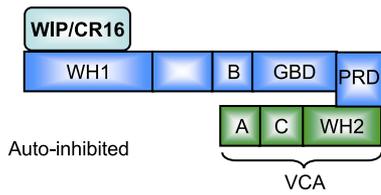
A) THE WISKOTT-ALDRICH SYNDROME PROTEINS. *I) Role in cell migration.* In the 1990s, microinjection experiments showed the specificity of Rho-GTPases in the reorganization of actin assembly in response to signaling, establishing that Rac1, Cdc42, and RhoA induce the formation of ruffles, filopodia, and stress fibers, respectively (152). The Wiskott-Aldrich syndrome proteins (WASP) were then identified as Arp2/3 activators that control actin assembly downstream of Cdc42 and Rac1. This family of proteins includes the hematopoietic WASP, the ubiquitous N-WASP (neural-WASP), and SCAR (suppressor of cAMP receptor)/WAVE (WASP-family verprolin homology protein) isoforms 1, 2, and 3. These proteins are involved in the regulation of actin dynamics in a variety of cellular processes including endocytosis, phagocytosis, cell migration, intracellular traffic, and internalization and propulsion of pathogens (see Refs. 212, 221 for reviews).

It was initially found that the function of N-WASP in cell migration was restricted to filopodia formation in response to Cdc42 (142), whereas WAVE isoforms (see sect. II C5B) were responsible for lamellipodium protrusion in response to Rac1 (143). However, N-WASP $-/-$ fibroblasts form filopodia in response to Cdc42 (127) (see sect. II D1). Moreover, the growing number of reports describing the localization of N-WASP at the leading edge of lamellipodia in MDCK, MDBK, myogenic, and carcinoma cells (13, 96, 117, 128) suggested a role for N-WASP in lamellipodial dynamics. In agreement with these observations, the siRNA depletion of N-WASP in MDBK cells inhibits cell migration (13). Nevertheless, in fibroblasts of N-WASP knockout mice (207) and in HeLa (85) and *Drosophila* S2 cells (182) depleted of N-WASP or WASP by RNAi, the formation of lamellipodia was not affected. In these cells and others, many studies demonstrated that the formation of lamellipodia is controlled by WAVE isoforms (85, 182, 209, 213, 254, 255). Finally, the RNAi depletion and the transfection of dominant negative forms of N-WASP or WAVE2 in myogenic cells reduced both the formation of lamellipodia and cell migration (96). Although it seems that WAVE isoforms play a more important role than N-WASP in cell migration, these observations suggest that the relative contribution of N-WASP and WAVE isoforms depends on cell type.

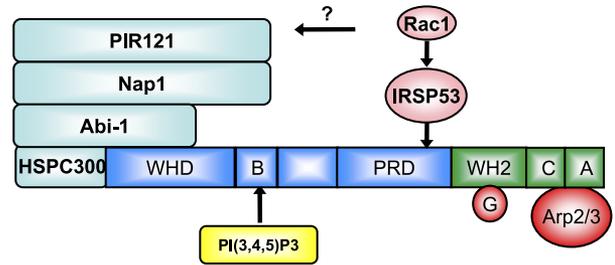
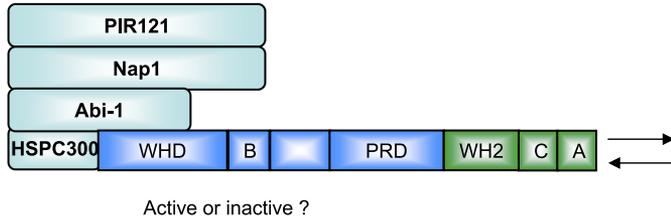
II) Regulation of WASP/N-WASP. *A) Activation of WASP/N-WASP.* N-WASP is a modular protein that contains a NH₂-terminal WASP homology 1 domain (WH1), followed by a basic region (B), a GTPase binding domain (GBD), a proline-rich domain (PRD), and a COOH-terminal catalytic domain (VCA or WA). The VCA domain is composed of two WASP homology 2 domains (WH2), a connector region (C), and an acidic domain (A) (Fig. 4). In vitro Cdc42 activates N-WASP, which in turn stimulates the ability of Arp2/3 to nucleate actin filaments (56, 184). N-WASP is autoinhibited by an intramolecular contact between the GBD domain and the VCA domain. The binding of Cdc42-GTP and PIP₂ to the GBD and the basic domains, respectively, activate N-WASP synergistically. Cdc42 and PIP₂ disrupt the intramolecular interaction to unmask the VCA domain (100, 168, 183). The exposed VCA domain binds Arp2/3, G-actin, and a filament barbed end as substrates to catalyze the formation of a branched filament (see sect. II C3). In addition to Cdc42, the Rho GTPase Rac1 activates N-WASP (225). The proline-rich region of N-WASP is a target for SH3 domain-containing proteins like Grb2, Nck, or Abi1 (31, 85, 185) that activate N-WASP.

VASP, which is localized along the leading edge of protruding lamellipodia (189), interacts with the proline-rich region of WASP but not N-WASP (34). This VASP binding motif found in WASP is conserved in WAVE1 and WAVE2. In vitro, VASP enhances the propulsion of WASP-coated beads in a minimum motility medium (34). Simi-

WASP / NWASP



SCAR / WAVE



Cortactin

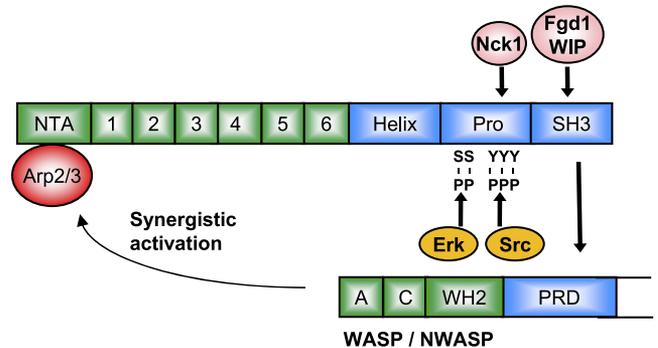
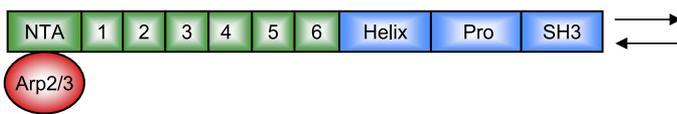


FIG. 4. Regulation of Arp2/3 activators. *Top:* WASP and N-WASP are modular proteins that contain a NH₂-terminal WASP homology 1 domain (WH1), followed by a basic region (B), a GTPase binding domain (GBD), a proline-rich domain (PRD), and a COOH-terminal catalytic domain (VCA). The VCA domain is composed of a WASP homology 2 domain (WH2), a connector region (C), and an acidic domain (A). N-WASP contains two WH2 domains. WASP and N-WASP (not shown) exist in an auto-inhibited conformation in which the VCA domain (green) is occluded by an intramolecular interaction. The binding of WIP (WASP interacting protein) or its homolog CR16 (corticosteroids and regional expression 16) to the WH1 (WASP homology 1) domain of WASP and N-WASP maintains this inactive state. A variety of ligands synergistically activate WASP and N-WASP by disrupting the intramolecular interaction to expose the COOH-terminal domain that binds and activates the Arp2/3 complex. The ligands include phosphatidylinositol 4,5-bisphosphate (PIP₂) that binds the B domain; the RhoGTPases Cdc42, Rac1, and TC10 that bind to the GBD; IQGAP1 that binds to a region encompassing the B and GBD domains of N-WASP; and several SH3 domain-containing proteins that interact with the central PRD (for a complete list, see Ref. 221). The phosphorylation of one tyrosine by Src and two serines by casein kinase II (CKII) also contribute to the activation of WASP. *Middle:* WAVE1, -2, and -3 are modular proteins that contain an NH₂-terminal WHD domain (WAVE homology domain) followed by a basic region (B), a proline-rich domain (PRD), and a COOH-terminal catalytic domain (VCA). WAVE1, -2, and -3 are part of a stable complex with four other proteins including Nap1 (Nck-associated protein), PIR121 (p53-inducible mRNA), HSPC300 (hematopoietic stem progenitor cell 300), and Abi1 (Abl-interactor 1). It was initially proposed that the complex is inactive and that Rac1 dissociates the active HSPC300-WAVE subcomplex. It was also proposed that the complex is active and that Rac1 does not dissociate it. In this model, Rac1 would only target the complex to the plasma membrane. The protein Irsp53 (insulin receptor substrate protein 53) has been shown to activate WAVE2 in the presence of Rac1 and phosphatidylinositol 3,4,5-trisphosphate (PIP₃). *Bottom:* cortactin is a modular protein that consists of a NH₂-terminal acidic domain (NTA) that binds the Arp2/3 complex, tandem repeats (1–6) that bind actin filaments, an α -helical region, a proline-rich domain (PRD), and a COOH-terminal SH3 domain. Cortactin displays a weak activity that is enhanced by the binding of WIP or Fgd1 to its COOH-terminal SH3 domain. This SH3 domain also binds and activates N-WASP, resulting in a synergistic activation of Arp2/3 by cortactin and N-WASP. Cortactin is phosphorylated on two serines/threonine kinases including Erk and three tyrosines by tyrosine kinases including Src.

larly, VASP interacts with the Arp2/3 activators ActA from *Listeria* and accelerates the propulsion of *Listeria* and ActA-coated beads by increasing the rate of dissociation of the branched filament from ActA (193). Whether this activity corresponds to the role of VASP in lamellipodia

extension remains to be established. More recently IQGAP1, a protein that has multiple binding partners including F-actin, microtubule binding proteins (CLIP-170 and APC), adherens junctions proteins (E-cadherin and β -catenin), Rho GTPases (Cdc42 and Rac1), and calmod-

ulin (26) was identified as a novel activator of N-WASP (13, 117). IQGAP1 links the N-WASP-Arp2/3 pathway to the fibroblast growth factor (FGF) receptor, FGFR1 (13) (Fig. 4).

Recent findings identified a mechanism by which WASP proteins may remain activated after signaling is switched off. Torres and Rosen (227) demonstrated that the activation of WASP by Cdc42 unmasks the residue Y291 of WASP allowing its phosphorylation by Src. This phosphorylation prevents the autoinhibitory interaction and maintains WASP in its active conformation after dissociation of Cdc42 (227) (Fig. 4). Interestingly, Cory et al. (42) also reported that casein kinase II phosphorylates two serines (S483 and S484) located in the VCA domain of WASP. In contrast to the previous report, the authors show that this phosphorylation is constitutive and does not require the previous activation of WASP. This phosphorylation increases the affinity of WASP for Arp2/3 and results in a higher stimulation of actin assembly both *in vitro* and *in vivo* (42) (Fig. 4). Altogether these two studies provide an example of molecular memory in cell signaling.

B) Inhibition of WASP/N-WASP. The basal activity of N-WASP observed *in vitro* is not compatible with site-directed actin assembly in response to signaling, suggesting that WASP proteins must be inhibited in resting cells. WIP (WASP interacting protein) and the homolog CR16 (corticosteroids and regional expression 16), which interact with both WASP and N-WASP (138, 175), inhibit N-WASP *in vitro* (138) (Fig. 4). However, WIP is necessary for actin-based processes including Cdc42-dependent extension of filopodia in fibroblasts and immunological synapse formation in lymphocytes (7, 138). To explain these apparent discrepancies, several hypotheses have been proposed. First, in lymphocytes, WASP is part of a tetrameric complex ZAP-70-CrkL-WIP-WASP. TCR activation leads to WIP phosphorylation and dissociation of the complex, allowing WASP to activate the Arp2/3 complex (194). Second, TOCA-1 can only activate N-WASP in complex with WIP (81). Hence, WIP would keep WASP/N-WASP inactive in the cytoplasm and control its localized activation.

III) Regulation of WAVE isoforms. SCAR (suppressor of cAMP receptor)/WAVE (WASP-family verprolin homology protein) isoforms 1, 2, and 3 were identified as WASP-related proteins. WAVE proteins are thought to be the major activators of Arp2/3 at the leading edge of lamellipodia downstream of Rac1 (143). The regulation of SCAR/WAVE proteins is very different from that of N-WASP and WASP described above. In contrast to WASP/N-WASP, SCAR/WAVE proteins are not autoinhibited, and they are not direct targets of Rho-GTPases since they do not contain any GBD domain. WAVE1, -2, and -3 are part of a stable complex with four other proteins including Nap1 (Nck-associated protein), PIR121 (p53-inducible

mRNA), HSPC300 (hematopoietic stem progenitor cell 300), and Abi1 (Abl-interactor 1) (55, 211). Nap1 and Abi1, which form the core of the complex, recruit PIR121 and the subcomplex HSPC300-WAVE, respectively (63). Whether and how the subunits of the complex activate or inhibit WAVE isoforms is still controversial. Eden et al. (55) first suggested that the WAVE1 complex isolated from bovine brain is inactive, and binding of Rac1 or Nck promotes the dissociation of Nap1, PIR121, and Abi1 from the subcomplex WAVE1-HSPC300, allowing WAVE1 to activate the Arp2/3 complex (55). Recent studies challenged this model and showed that the recombinant WAVE2 complex is active. Moreover, Rac1 did not dissociate the complex (86, 209; see Ref. 212 for a review). An alternative model for WAVE2 activation proposed that the protein IRSp53 binds Rac1 and WAVE2 to form a Rac1/IRSp53/WAVE2 complex that induces membrane ruffling (144)(Fig. 4).

B) CORTACTIN. Cortactin, an actin filament-binding protein, is an important link between signaling pathways and the actin cytoskeleton in a variety of cellular processes including cell migration, cell-cell adhesion, and endocytosis (see Ref. 43 for a review). In cells, cortactin is enriched at the leading edge of lamellipodia where it colocalizes with actin and Arp2/3 (245). Although the importance of cortactin in cell migration is generally accepted, its precise role in lamellipodial dynamics is still unclear (discussed in Ref. 43).

Cortactin is a modular protein that consists of a NH₂-terminal acidic domain that binds the Arp2/3 complex, tandem repeats that bind actin filaments, and a COOH-terminal SH3 domain. The acidic domain and the tandem repeats are sufficient to stimulate the Arp2/3 complex (230, 243). Although this activity is very low compared with WASP proteins, the binding of WIP and Fgd1 to the SH3 domain of cortactin greatly enhances the activation of the Arp2/3 complex (101, 103). Interestingly, cortactin and N-WASP cooperate tightly to stimulate actin assembly through the Arp2/3 complex. Cortactin and N-WASP binding to Arp2/3 are not mutually exclusive and activate the complex synergistically (242). More recently, two independent studies showed that the SH3 domain of cortactin binds and activates N-WASP to stimulate cell migration (108, 137).

A large body of evidence indicates that cortactin phosphorylation plays an important role in actin remodeling in a variety of physiological and pathological processes (43). Cortactin is phosphorylated by tyrosine kinases including Src, Fyn, Syk, and Fer and by serine/threonine kinases including Erk and PAK (43). *In vitro*, the phosphorylation of cortactin by Erk enhances the activation of N-WASP (137). Several effects of cortactin phosphorylation by Src have also been described. First, cortactin phosphorylation by Src prevents Erk-phosphorylated cortactin from activating N-WASP (137). Second, in

a reconstituted assay, cortactin phosphorylation by Src enhanced Arp2/3-mediated actin assembly in the presence of Nck1, N-WASP, and WIP (223)(Fig. 4). The relative contribution of these activities *in vivo* remains to be determined.

C) SPECIFICITY OF ARP2/3 ACTIVATORS. The density of the branched actin network, which controls the mechanical properties of the actin array, contributes to change the cell shape in many actin-based processes such as lamellipodium protrusion, endocytosis, and phagocytosis. Each Arp2/3 activator has distinct activities and regulation that are well-adapted to achieve specific cellular functions. WASP proteins harbor different levels of Arp2/3 activation that depend on the number of acidic amino acids at the COOH terminus. Among the WASP proteins, N-WASP is 16-fold more active than WASP and 68-fold more active than Scar1/WAVE1 in solution (262). Although cortactin is a much weaker activator than WASP proteins, it activates N-WASP and stabilizes the branched filaments, thus contributing to the density of the actin array. In addition to these intrinsic differences, N-WASP, cortactin, and WAVE also regulate cell shape differently by coupling actin assembly to different membrane curvature proteins. For example, N-WASP associates with BAR domain-containing proteins such as endophilin (159) and EFC domain-containing proteins such as syndapin-I (99) that are thought to sense or promote membrane invagination in endocytosis. Cortactin also interacts with dynamin that is involved in membrane invagination and scission during endocytosis (196). Interestingly, WAVE2 interacts with the IMD domain containing protein Irs53 (144). In contrast to BAR and EFC domains, the IMD domain has an opposite structure and induces membrane protrusion. These observations suggest that membrane-deforming proteins and actin assembly synergize to induce membrane protrusion (139, 221).

A full description of the relative contribution of these pathways will require an in-depth cell biological dissection of the spatial and temporal localization of Arp2/3 regulators. The dissection of the sequential roles of several Arp2/3 activators in yeast already showed that they are involved in different steps of endocytosis (214). The development of biosensors to probe the activity of Arp2/3 (69) and WASP proteins (128) will also help to establish the specificity of these different pathways.

D. Filopodia Formation

In migrating cells, lamellipodia and filopodia are overlapping but distinct actin networks with different organization and dynamic properties. How the cell manages to build such different structures in the same cellular

environment is a crucial issue in the coordination of protrusions in cell migration. The past years have seen a growing number of molecular models explaining how filopodia emerge from the lamellipodial actin network (see Ref. 59 for a review).

1. Description and first molecular models

During cell migration, cells extend fingerlike protrusions called filopodia beyond the leading edge of protruding lamellipodia to sense the environment (Fig. 1). These protrusions contain 15–20 parallel filaments tightly packed into a bundle with their barbed ends facing the membrane (118). Extension and retraction of filopodia are controlled by actin assembly at the tip and retrograde flow, respectively (60, 132, 145, 155).

Nobes and Hall (152) established that the Rho-GTPase Cdc42 controls the formation of filopodia. The fact that N-WASP potentiates the ability of Cdc42 to induce filopodia (142) suggested that the stimulation of Arp2/3 was involved in the initiation of these structures. However, N-WASP null fibroblasts extend normal filopodia in response to Cdc42 (127), Arp2/3 is excluded from filopodia (218), and the branched actin network nucleated by Arp2/3 is not compatible with the tight bundle of unbranched actin filaments found in filopodia. To conciliate these data, it was proposed that the formation of filopodia was the result of a local rearrangement of the lamellipodial branched actin network generated by Arp2/3 into parallel filaments by proteins like fascin (218, 232). However, this model does not include the constant nucleation of actin filaments that is required to balance the capping protein effect. The fact that VASP localizes at the tip of filopodia (218) and causes the dissociation of capping proteins from the barbed ends (12) led the authors to propose a model in which VASP promotes filopodia extension by protecting the barbed ends of actin filaments generated by Arp2/3 from capping. This model was further supported by the observation that capping protein depletion induces the formation of filopodia (141). However, the observation that filopodia form normally in Arp2/3-depleted cells ruled out this model (208). Also, the inhibition of barbed end capping by VASP was not confirmed (193, 197). So far, the link between the known biochemical activities of VASP (actin filament binding, bundling, and nucleation) and its localization at the tip of filopodia is not understood.

Additional models ascribed a key role for the Cdc42 effector Irs53 in the formation of filopodia (54, 109). The protein Irs53 activated by Cdc42 would recruit Mena to bundle actin filament and promote the formation of filopodia (109). In addition, the ability of the Irs53-Eps8 complex to bundle actin filaments is required for the Cdc42-dependent formation of filopodia (54). Although these mechanisms account for the structural reorganization of actin

filaments downstream of Cdc42, they do not explain the fast elongation of actin filament in filopodia. The newly discovered formins are good candidates to nucleate parallel actin filaments at the tip of filopodia.

2. Role of formins in filopodia formation

Formins are involved in many different cellular processes including cytokinesis, endocytosis, filopodia formation, cell polarity, cell-cell adhesion, and cell-matrix adhesion (see Refs. 71, 104 for reviews).

Several observations demonstrate the role of formins in the formation of filopodia downstream of Rho-GTPases in both mammalian cells and *Dictyostelium*. The diaphanous-related formin mDia2 localizes at the tip of filopodia (165, 166). The expression of a constitutively active mDia2 induced the formation of filopodia (235), whereas microinjection of anti-mDia2 antibodies or expression of a dominant negative form of mDia2 blocked actin reorganization and filopodia formation in response to Cdc42 in NIH 3T3 cells (166). A variety of Rho-GTPases appear to activate a given forming. For instance, Rif (Rho in filopodia) seems to play a more prominent role in the formation of mDia2-dependent filopodia than Cdc42 (165). Cdc42 may not be involved in targeting mDia2 to the tip of filopodia, but simply contributes to its activation (166). In *Dictyostelium*, dDia2 also localizes at the tip of filopodia and is required for their extension (199). The ability of dDia2 to induce filopodia also depends on the bundling activity of the F-actin binding domain of VASP (198).

Formins are modular proteins characterized by the presence of two conserved domains: formin homology 1 and 2 (FH1 and FH2). The FH1 domain contains polyproline repeats that interact with SH3 domains and profilin (58, 241). The FH2 domain nucleates actin filaments by stabilizing an actin dimer (106, 170, 171, 192).

Formins exist in an autoinhibited conformation in which the COOH-terminal DAD (diaphanous autoregulatory domain) interacts with the NH₂-terminal DID (diaphanous inhibitory domain) and masks the nucleating domain FH2 (1, 119). Biochemical and structural studies demonstrate that the binding of Rho-GTPases to the NH₂-terminal GBD domain present in most formin isoforms releases this autoinhibitory interaction (110, 119, 188).

In addition to its nucleating activity, the FH2 domain binds to the barbed end and acts as a "leaky capper" by slowing down the elongation and dissociation rates (170, 171, 266) without affecting the critical concentration. The resolution of the crystal structure of the FH2 domain of the yeast formin Bni1p revealed a "donut-shaped" structure corresponding to a flexible antiparallel dimer of elongated FH2 domains (252). The linker between the FH1 and FH2 domains that mediates the dimerization is required for the nucleating activity of the FH2 domain (202). In the presence of profilin, a single actin filament, whose

barbed end is bound to a formin (mDia1)-coated bead, grows rapidly by processive barbed end assembly for up to 4,000 consecutive steps. This remarkable observation demonstrates that profilin induces a switch in the function of the formin mDia1 from a leaker capper to a processive motor of actin assembly. In this process, mDia1 increases the rate constant for profilin-actin association to the barbed end by 15-fold (187). Such a processive activity requires a source of free energy. mDia1 is not a canonic motor itself but, in agreement with the definition of a molecular motor, it accelerates ATP hydrolysis associated with profilin-actin assembly to dissociate profilin from the barbed end allowing further elongation (186, 187) (Fig. 5). Although it was reported that several formins catalyze the processive growth of ADP-actin filaments in the absence of profilin (105), a theoretical model failed to accommodate these data using the published K_d for profilin binding to actin (231). The structure of TMR-actin in complex with the FH2 domain of Bni1p suggested a mechanism for formin processivity. In this structure, the filament sits in the center of the formin ring where each FH2 domain interacts with two actin subunits. On the basis of this structure, the formin dimer makes contact with the three terminal subunits at the barbed end, consistent with its nucleating function. One protomer of the formin dimer interacts with the first (terminal) and second subunits; the second protomer interacts with the second and third subunits. Release of one of the two bonds between the second actin subunit and one protomer has to be hypothesized to uncap the barbed end and allow processive growth (158) (Fig. 5). This dissociation could be mediated by ATP hydrolysis and the resulting profilin dissociation (186, 187). The helical structure of the filament implies a rotation of the formin around the barbed end. However, the absence of supercoils in tethered filaments emanating from an immobilized formin ruled out this possibility (107). Although a theoretical model has been proposed (201), this paradox is still not understood.

In a minimum motility medium containing ADF, profilin, ATP, and actin, mDia1-coated beads move at a much faster rate (20 $\mu\text{m}/\text{min}$) (187) than the Arp2/3-dependent N-WASP beads (2 $\mu\text{m}/\text{min}$; Ref. 249). This movement is further accelerated by gelsolin, but in contrast to Arp2/3-dependent movement, gelsolin poisons mDia1-coated beads resulting in a transient movement (187). These observations demonstrate that the activation of two different nucleation machineries (N-WASP-Arp2/3 and formins) at the plasma membrane, in the presence of the same pool of soluble proteins, is sufficient to generate two actin networks with different structural and dynamic properties. This may explain why lamellipodia and filopodia, which are characterized by different actin organization and dynamics, coexist in the same cells.

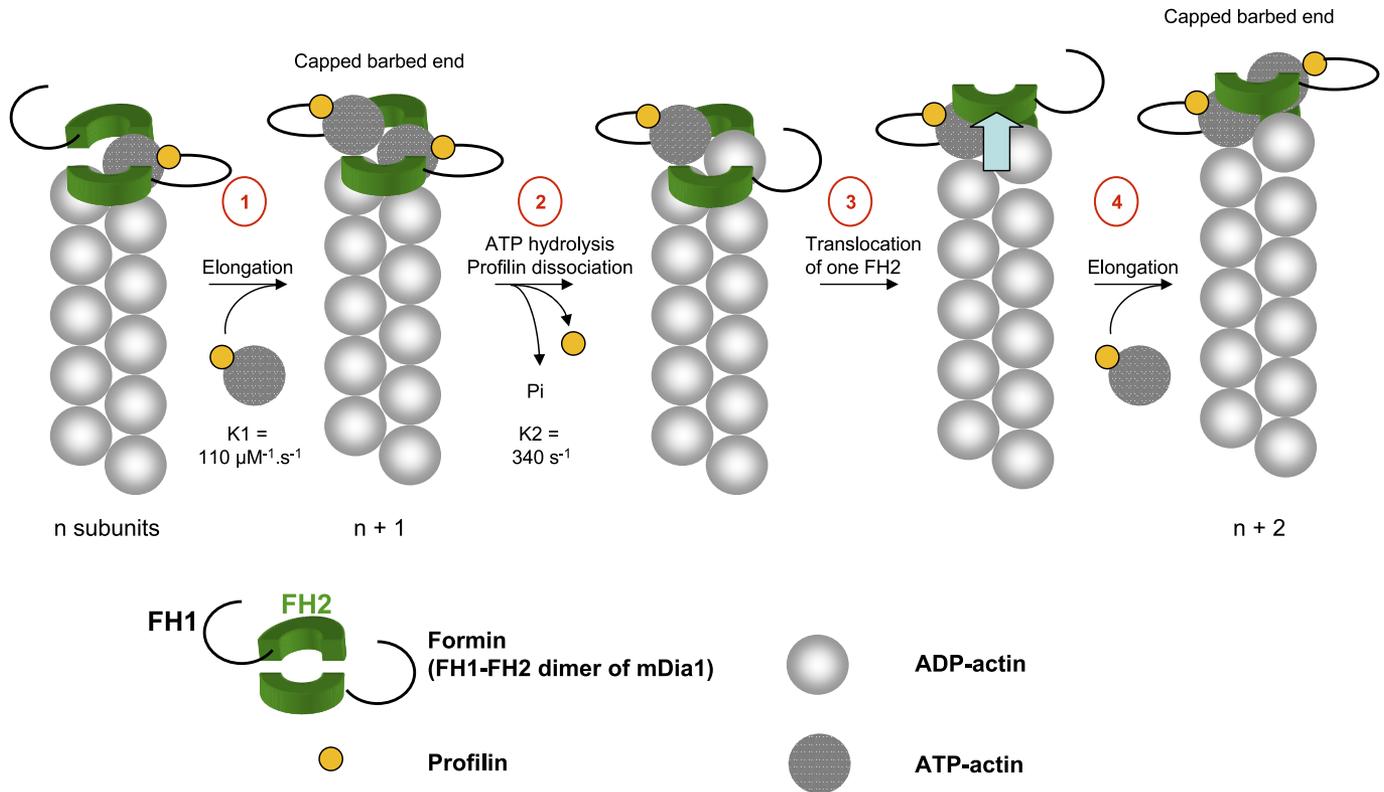


FIG. 5. Model for the processive assembly of profilin-actin by the formin mDia1. 1) The assembly of profilin-actin to a formin-bound barbed end results in a situation in which the two terminal actin subunits bind profilin and cap the barbed end. Each FH2 protomer of the mDia1 dimer makes two contacts with the terminal subunits at a barbed end; the first protomer interacts with the first and second subunits, and the second protomer interacts with the second and third subunits. 2) ATP hydrolysis induces the dissociation of profilin. 3) The translocation of one FH2 protomer releases one of the two actin-formin bonds. 4) Addition of the next profilin-actin subunit to the uncapped formin-bound barbed end.

III. ACTIN DYNAMICS AND CELL-MATRIX ADHESION

In order for cells to migrate rapidly, adhesions form at the leading edge and disassemble at the trailing edge. In these structures, adhesion is mediated by integrins, a large family of heterodimeric transmembrane receptors that connect the extracellular matrix to the actin cytoskeleton. Activation of integrins results in a variety of intracellular responses including the phosphorylation-dependent recruitment of signaling proteins. This part focuses on actin dynamics associated with cell-matrix adhesion. Therefore, the role of major signaling proteins such as the adaptor paxillin and the kinases Src and FAK is beyond the scope of this review (for reviews, see Refs. 25, 33, 229).

A. Adhesion Controls the Mechanical Coupling Between the Actin Cytoskeleton and the Substrate

Cell locomotion depends on the protrusion of the leading edge, the traction of the cell body, and the retrac-

tion of the tail. In these processes, cell matrix adhesion acts as a “molecular clutch” that controls the mechanical coupling between actin dynamics and the substrate (24, 83, 120, 145, 215, 216). When the clutch is engaged, the force generated by actin assembly at the leading edge of the lamellipodium is converted into protrusion (Fig. 6*B*). In contrast, when the clutch is disengaged, the slippage that occurs between the polymerizing actin network and adhesions increases the retrograde flow and decreases the protrusion rate (Fig. 6*A*). This molecular clutch also controls the transmission of the actomyosin contractile force applied on focal adhesions that enables the traction of the cell body and the retraction of the tail (Fig. 6, *A* and *B*). In addition to transmitting the force, cell-matrix adhesions behave as mechanosensors. The tension generated by the actomyosin contraction induces focal adhesion maturation during which the structure grows, recruits new actin binding proteins, and initiates actin assembly (see Refs. 244, 253, 261, 263 for reviews).

The molecular clutch engagement is determined by regulated molecular interactions at different levels: integrin-substrate, integrin-actin binding proteins, and actin filaments-actin binding proteins. Until recently, it was

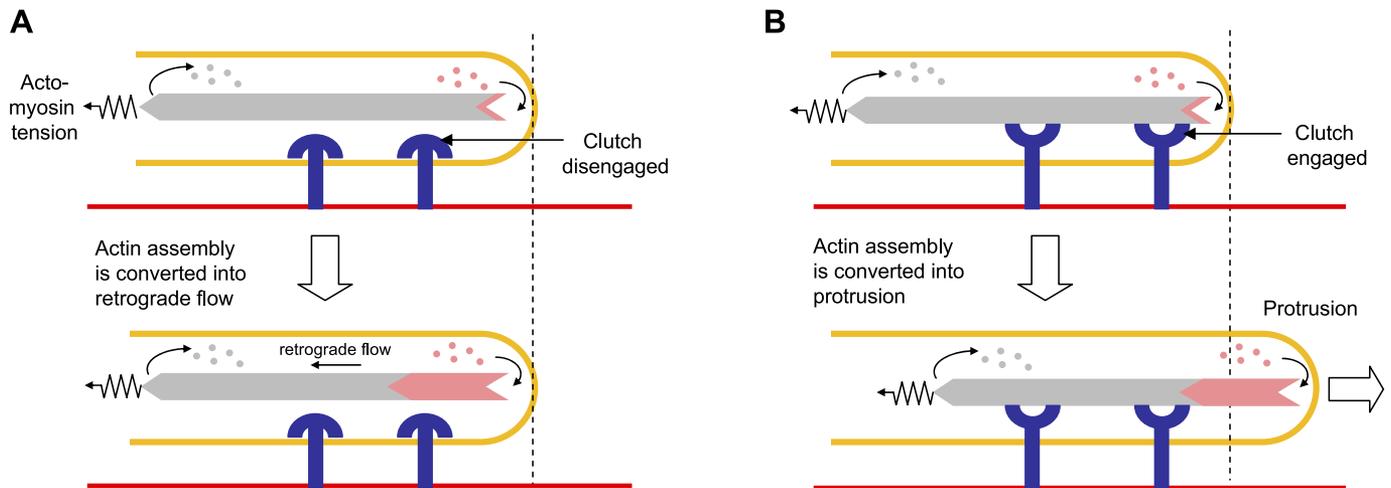


FIG. 6. Adhesion acts as a “molecular clutch” to convert the force generated by actin assembly into protrusion. In these panels, the actin network is represented in gray. The newly polymerized actin is represented in pink. *A*: the molecular clutch is disengaged; there is no connection between adhesions and the actin cytoskeleton. In this situation, no protrusion occurs because actin treadmilling is mainly converted into retrograde flow. *B*: the molecular clutch is fully engaged. The strong connection between the polymerizing actin network and the substrate results in membrane protrusion. Moreover, this connection enables the conversion of the acto-myosin tension into traction of the cell body and retraction of the tail.

difficult to measure the relative contribution of these interfaces and their individual components to the force transmission. A recent work that combined TIRFM and speckle fluorescence microscopy (SFM) revealed different degrees of correlated motions between actin binding proteins, integrin, and the retrograde flow of actin filaments in focal adhesions. This study showed that α -actinin displays the highest coupling; vinculin and talin motion was only partially coupled, whereas integrin was not coupled to actin filaments (83). Similar conclusions were derived from spatiotemporal image correlation spectroscopy (STICS) (24). These experiments suggested that vinculin and talin form transient linkages that enable a partial transmission of force to the substrate.

Although it is still difficult to predict the ability of known actin binding proteins to transmit the force, we can anticipate several possible mechanisms. The molecular clutch engagement should depend, in part at least, on the association and dissociation rates of a variety of actin binding proteins present in focal adhesions. In addition, the ability of several actin binding proteins to block or accelerate actin filament barbed end elongation may control the force transmission differently. For example, a true capping protein like tensin, which blocks actin filament barbed end elongation (see sect. III C 2c), is expected to provide a stable link and an efficient force transmission. In contrast, a nucleator and processive motor like the formin mDial that accelerates the elongation of actin filament barbed ends (see sect. II D 2) should compensate the actomyosin tension. Finally, the tension generated by stress fibers also depends on their mechanical properties. A protein like α -actinin that increases the stiffness of stress fibers by bundling actin filaments may facilitate the transmission of force. How these activities are integrated

and/or sequentially well orchestrated to finely tune the force transmission during focal adhesion maturation is not understood.

B. Description of Cell-Matrix Adhesions

Adhesion structures can be classified into focal complexes, focal adhesions, and fibrillar adhesions. The classification depends on their size, shape, intracellular localization, molecular composition, and dynamics (see Refs. 244, 253, 261, 263 for reviews). Focal complexes are highly dynamic nascent adhesions that disassemble and reassemble at the leading edge during protrusion in a process called adhesion turnover. Focal complexes appear as small dotlike structures of $1 \mu\text{m}^2$ at the cell periphery of spreading cells or at the leading edge of migrating cells. These structures are associated with a loose actin meshwork (190). Early focal complexes contain β_3 -integrin, talin, and paxillin. Vinculin, α -actinin, VASP, and FAK appear in late focal complexes (111, 190, 260, 261) (Fig. 7A). In slow moving cells, focal complexes that did not disassemble mature into focal adhesions (or focal contacts) of $2\text{--}5 \mu\text{m}$ long in response to RhoA signaling. Focal adhesions are more stable and display a slower turnover than focal complexes. They are located at the cell periphery and more centrally in less motile regions, associated with the end of stress fibers. These structures contain high levels of vinculin, talin, paxillin, zyxin, α -actinin, VASP, FAK, phosphotyrosine proteins, and integrin $\alpha_V\beta_3$ (261) and actopaxin (150) (Fig. 7B). Fibrillar adhesions that arise from focal adhesions are elongated structures associated with fibronectin fibrils and located more centrally in cells. In contrast to focal

adhesions, fibrillar adhesions are not associated with stress fibers but only thin actin cables and contain high levels of tensin and $\alpha_5\beta_1$ -integrin, only traces of paxillin, and no vinculin (Fig. 7C). The elongation of fibrillar adhesions depends on the deformability of the extracellular matrix (264).

In addition to the classical focal complexes and focal adhesions, several cell types form unique adhesion structures. Podosomes and invadopodia are two actin-rich structures that combine adhesion and matrix degradation properties in physiological and pathological invasion processes (for recent reviews, see Refs. 28, 121).

C. Actin Dynamics Associated With Adhesions

1. Initiation of actin assembly at adhesion sites

Although the nucleation of actin filaments in lamellipodia and filopodia is now well understood, less is known about the initiation of actin assembly at adhesion sites.

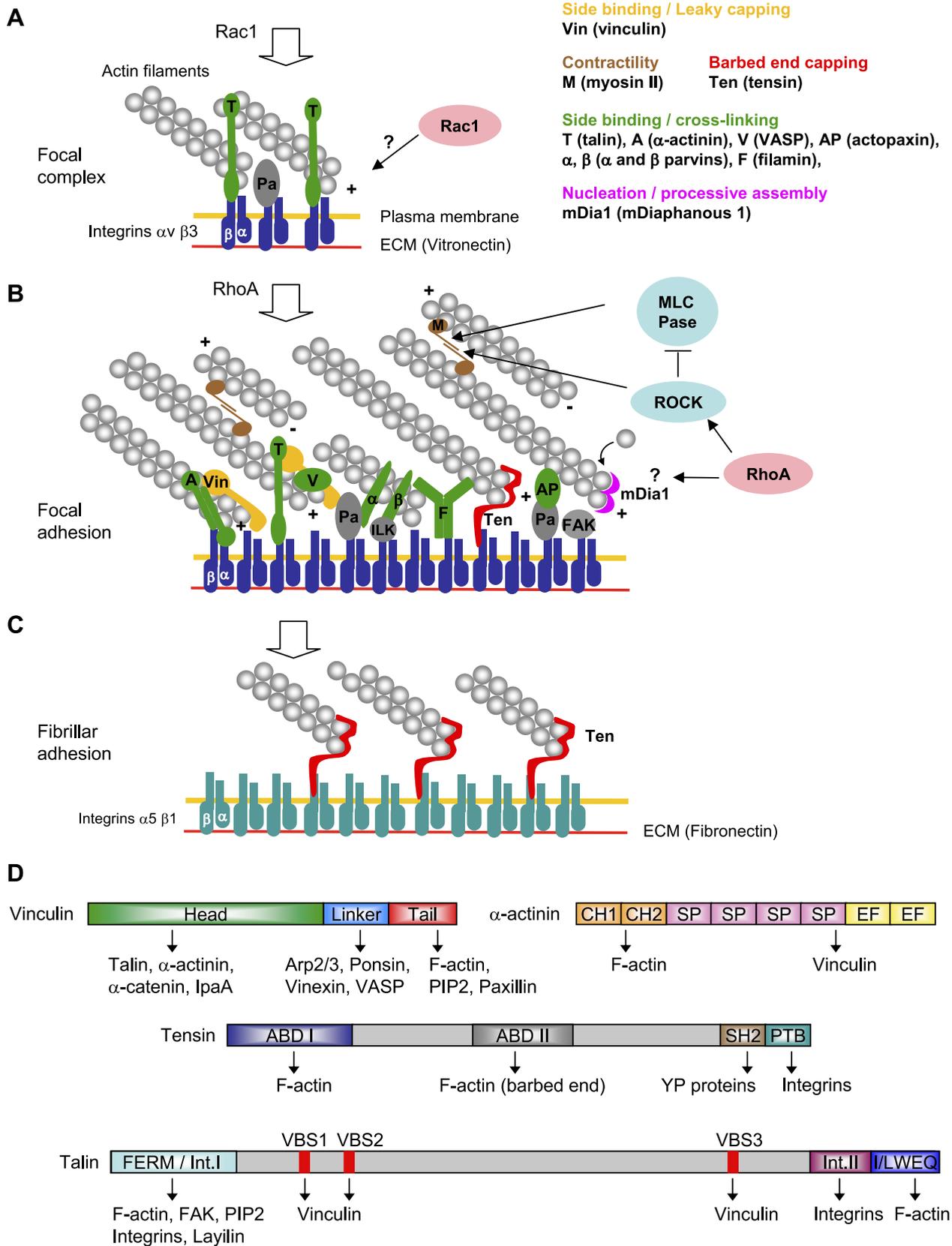
In cells, microinjected rhodamine-labeled monomeric actin incorporates first in focal complexes at the cell periphery and slightly later in stress fibers (68). Live cell microscopy also shows that actin structures appear as small spots at the leading edge and extend into stress fibers (239). Whether these first observations correspond to the elongation of captured preexisting actin filaments or nucleation of actin filaments or both is still unclear. The following sections give an overview of molecular pathways that have been proposed to initiate actin assembly in early focal complexes and mature focal adhesions.

A) INITIATION OF ACTIN ASSEMBLY AT FOCAL COMPLEXES. Extensive research has been carried out to identify the molecular pathways linking integrins to actin assembly at adhesions sites. Among the downstream targets of Rac1 that control the formation of focal complexes, recent findings identified the Arp2/3 complex as a potential actin nucleator in nascent focal complexes. Arp2/3 has been colocalized with vinculin at the leading edge in focal complexlike structures. These authors also showed that vinculin interacts directly with Arp2/3. In addition, cells transfected with a vinculin point mutant, which does not interact with Arp2/3, showed defects in lamellipodium extension and cell spreading (50). However, vinculin does not activate Arp2/3 *in vitro* (50), and the known activators of Arp2/3 (N-WASP, WAVEs, and cortactin) have not been localized at focal complexes. Although the mechanism of actin filament nucleation at focal complexes remains elusive, several formins regulated by Rac1 appear as interesting candidates. VASP, which displays a weak nucleating activity *in vitro* (112) and localizes to focal complexes (176), is also a good candidate. Finally, the study of multiprotein complexes made of early components of focal complexes like talin, vinculin, and others could reveal unanticipated nucleating activities.

B) FORMATION OF STRESS FIBERS ASSOCIATED WITH MATURE FOCAL ADHESIONS. Stress fibers are contractile bundles of actin and myosin associated with focal adhesions. These structures are distributed in three classes: ventral stress fibers that are located at the ventral cell surface and associated with focal adhesions at both ends, dorsal stress fibers that are associated with focal adhesions at one end, and transverse arcs that are not associated with focal adhesions (82, 205) (Fig. 1). The force applied by these structures to focal adhesions enables the retraction of the rear of the cell. Although the contractile actomyosin array is organized differently in other cell types, its function remains the same (204). Stress fibers contain antiparallel actin filaments (45), myosin II (62), and several actin filament binding proteins, including α -actinin (113). Stress fibers and focal adhesions are functionally interactive structures. Focal adhesions initiate the elongation of stress fibers, and the tension generated by stress fibers enhances the growth of mechanosensitive focal adhesions. Accordingly, the inhibition of myosin II-mediated contractility using different strategies affects both stress fibers and focal adhesion formation (14, 38, 76, 95, 163, 190, 233).

The formation of stress fibers connected to focal adhesions involves the organization of contractile actomyosin bundles and actin assembly in response to RhoA (177). First, the GTP-bound RhoA activates ROCK (Rho-kinase) that phosphorylates the myosin II light chain (MLC) and inhibits the MLC phosphatase. The phosphorylated myosin II assembles into myosin filaments and associates with actin filaments to form contractile stress fibers (102) (Fig. 7B). The induction of contractility by the RhoA-ROCK pathway is required for the formation of focal adhesions (5) and seems to be the major function of this pathway since the inhibition of RhoA is bypassed by an external force (178).

The myosin II-driven contractility of stress fibers implies that these structures are composed of actin filaments with barbed ends facing focal adhesions and actin filaments with reverse polarity. If it becomes clear that the first population of filaments is nucleated or recruited by focal adhesion proteins, the origin of the second population is unclear. The constant ruffling at the cell front and the lateral flow could bring preexisting actin filaments with opposite polarity that associate into stress fibers through myosin (204). Accordingly, the microinjection of rhodamine-labeled phalloidin revealed the recruitment of existing filaments by stress fibers (238). Alternatively, the binding of bipolar myosin mini-filaments to the first population of filaments may initiate polymerization of filaments of opposite polarity. To understand how stress fibers are assembled, a detailed analysis of their structure, in terms of number of free and capped barbed ends and pointed ends, would be useful.



In addition to the actomyosin contractility, several lines of evidence suggest that RhoA activates actin assembly through the actin nucleating factor mDia1. 1) The simultaneous expression of ROCK and formin mDia1 recapitulates the formation of focal adhesions and stress fibers (240). 2) In cells where RhoA is inhibited, the transfection of constitutively active mutants of mDia1 is sufficient to restore the growth of focal adhesions in response to an external force. 3) Inhibition of mDia1 leads to a decrease in the number of stress fibers (226, 240). 4) The autoinhibitory domain of mDia1 inhibits actin assembly induced by purified integrin complexes *in vitro* (29). 5) The siRNA knock down of mDia1 affects both the dynamics and the morphology of dorsal stress fibers (82). However, the total depletion of mDia1 does not abolish the formation of stress fibers but only decreases their elongation rate (82), suggesting that the major function of mDia1 is probably not to nucleate the filaments but to link their processive growth to focal adhesions (187). Since focal adhesions are the result of the maturation of focal complexes, it is also possible that the actin filaments present in stress fibers have been nucleated earlier in focal complexes.

2. Function and regulation of major actin binding proteins

A) VINCULIN. Vinculin is a ubiquitously expressed actin filament binding protein that localizes in late focal complexes and focal adhesions. Vinculin is considered as a tumor suppressor (181) because vinculin null fibroblasts are less adherent and show increased cell migration (251), whereas overexpression of vinculin inhibits cell migration (180). Interestingly, in vinculin null fibroblasts, the number and the size of focal adhesions are reduced, whereas their turnover is faster. This last observation together with the high level of vinculin in focal adhesions suggest that this protein plays a key role in the stabilization of the adhesive structure during the maturation of focal complexes into focal adhesions.

This large protein of 1,066 amino acids (116 kDa) is made of a NH₂-terminal globular head (Vh) and a COOH-terminal elongated tail (Vt) linked by a central polypro-

line-rich domain (Fig. 7D). Vinculin is regulated by an intramolecular interaction in which the head interacts with the tail (94). This intramolecular interaction masks the actin filament binding domain located in the tail. The binding of several ligands including talin, α -actinin, and the *Shigella* protein IpaA to the head domain of the autoinhibited vinculin disrupts the intramolecular interaction and exposes the cryptic actin binding domain. This process is defined as “activation” of vinculin (see Refs. 47, 265 for reviews).

Vinculin binding to actin filaments is activated by a peptide corresponding to one of the numerous vinculin binding sites (VBS) of talin (11, 67). The recent resolution of vinculin crystal structure in its active and inactive states (9, 89) revealed that the head domain undergoes dramatic structural changes (an helical bundle conversion) upon talin VBS binding (89). However, the concentration of talin peptide at which 50% of vinculin is bound to F-actin is $\sim 500 \mu\text{M}$ in a cosedimentation assay (10) because the head-to-tail binding ($K_d = 20\text{--}50 \text{ nM}$ between isolated domains and $<1 \text{ nM}$ for the full-length protein) is stronger than the binding of the talin peptide to the head ($K_d = 400 \text{ nM}$) (9). The very low apparent affinity of talin for full-length vinculin suggests that *in vivo* the activation of vinculin is a complex process in which several ligands cooperate to activate vinculin (9). In support to this hypothesis, the disruption of the head-to-tail interaction is a prerequisite for the formation of vinculin-talin complexes in cells (41). In addition, the binding of PIP₂ to the tail domain induces a conformational change in vinculin that exposes the talin binding site (66). Similarly, the binding of VASP to a FPPP motif in the linker region increases the affinity of the tail domain for PIP₂ (84). PIP₂ and F-actin binding to vinculin are mutually exclusive (210), suggesting that other combinations of ligands induce actin filament binding to vinculin. Actin filaments also contribute to the activation of vinculin (35). The cryo-EM structure of the tail domain bound to actin filaments revealed the presence of two actin binding sites; one is partly masked by the intramolecular interaction with the head domain, whereas the second is exposed, opening the possibility for actin to cooperate with other ligands to fully unfold

FIG. 7. Actin binding proteins in focal complexes, focal adhesions, and fibrillar adhesions. *A*: focal complexes are formed in response to Rac1 signaling. Nascent focal complexes contain integrin $\beta 3$, talin (Ta), and Paxillin (Pa). In this simplified representation, VASP (V), tensin (Ten), FAK, α -actinin (A), and vinculin (Vin) are absent from focal complexes and appear only in focal adhesions. These proteins are actually present in late focal complexes. *B*: the maturation of focal complexes into focal adhesions occurs in response to RhoA signaling. In this process, RhoA stimulates the contractility of stress fibers by activating ROCK. ROCK phosphorylates the myosin light chain and inhibits the myosin light-chain phosphatase (MLC Pase). Although mDia1 has never been localized in focal adhesions, several lines of evidence suggest that it contributes to the nucleation and elongation of actin filaments downstream of RhoA in these structures. Focal adhesions contain integrin $\beta 3$ and a variety of actin binding proteins: the side binders and/or cross-linkers filamin (F), α -actinin (A), talin (Ta), vinculin (Vin), α - and β -parvins (α , β), actopaxin (AP), the leaky capper vinculin (Vin), and the capping protein tensin (Ten). *C*: fibrillar adhesions contain high levels of tensin. *D*: domains and binding partners of vinculin, talin, tensin, and α -actinin. CH, calponin homology; SP, spectrin repeat; EF, EF hand motif; ABD, actin binding domain; SH2, Src homology 2; PTB, phosphotyrosine binding domain; FERM, four-point-one, ezrin, radixin, moesin; Int, integrin binding domain; VBS, vinculin binding site; YP proteins, tyrosine-phosphorylated proteins.

vinculin in its active state (91). However, Bois et al. (19) challenged this combinatorial mechanism and proposed that the high-affinity binding of VBS peptides of talin or α -actinin is sufficient to activate vinculin (19).

The regulation of actin binding to vinculin has been very well documented, but little is known about the function of this interaction. The vinculin binding protein IpaA from the bacteria *Shigella*, which is injected in the host cell during bacteria entry, provides valuable insights into the physiological function of vinculin. In vivo, IpaA induces a vinculin-dependent actin depolymerization (22, 228). In vitro, the IpaA-vinculin complex prevents actin assembly (22). The binding of the COOH-terminal domain of IpaA to the head domain of vinculin is sufficient to unmask the tail domain, promote actin filament binding, and partially cap actin filament barbed ends (174). Structural studies demonstrate that the COOH terminus of IpaA mimics the activation of vinculin by talin (74, 90), suggesting that the “leaky capping” of actin filament barbed ends at focal adhesions could represent the cellular function of activated vinculin. As mentioned earlier, the turnover of vinculin at focal adhesions is partially coupled to that of actin filaments, suggesting a role for vinculin in the mechanical coupling between actin filaments and the substrate (24, 83). Whether the leaky capping function of vinculin contributes to the force transmission in focal adhesions is an open issue.

B) TALIN. Several studies suggest that talin is an important component of adhesions that links the integrin receptors to the actin cytoskeleton. In *C. elegans*, both the RNAi knockdown of talin and integrin knockout affect cytoskeleton organization and cell migration (44). In *Drosophila*, talin is essential for integrin function (27). In mice, talin knockout provokes cell migration defects, leading to an early embryonic lethality (147). The study of talin $-/-$ ES cells, the microinjection of dominant negative forms and antibodies as well as the RNAi knockdown revealed that talin is essential for focal adhesion assembly (2, 77, 134, 154, 169). Force measurement experiments demonstrated a requirement of talin for the 2 pN bond corresponding to the integrin-actin linkage in nascent focal complexes (92). Talin is also required for the reinforcement of the integrin-actin linkage and the assembly of adhesions in response to an external force (64).

Talin is a large protein (270 kDa) made of a small globular head domain and a large elongated rod domain that associates into an antiparallel homodimer. The head domain contains a FERM (four-point-one, ezrin, radixin, moesin) domain that directly binds integrin α IIb, β_1 and β_3 cytoplasmic tails, FAK, layilin, PIP₂, and actin filaments. The binding of PIP₂ to talin induces a conformational change of the protein and enhances the interaction between the cytoplasmic tail of β_1 -integrin and talin (133). The rod contains an I/LWEQ actin filament binding domain. This domain also mediates dimerization and con-

tains multiple vinculin binding sites (see Refs. 47, 149 for reviews) (Fig. 7D).

Talin connects actin filaments to focal adhesions directly through the I/LWEQ and the FERM domains and indirectly through the vinculin binding sites. The I/LWEQ domain is regulated by an intramolecular interaction in which an upstream α -helix (USH) masks the actin binding site (200, 257). So far, the mechanism of activation of the I/LWEQ domain in focal adhesions, the respective importance of the two actin binding domains of talin, and the synergy between talin and vinculin in the anchorage of filament barbed ends at focal complexes and focal adhesions are challenging issues.

The role of talin is not only to link the cytoplasmic tail of β -integrins to the actin cytoskeleton but also to increase the affinity of the extracellular domain of integrin for the extracellular matrix (220, 222). In *Drosophila*, a mutation in the integrin binding site located in the talin head domain reduced integrin activation but had no effect on the ability of talin to connect integrin to the actin cytoskeleton, suggesting that these two functions of talin are regulated by distinct mechanisms (222).

C) TENSIN. This modular protein of 1,744 amino acids binds to the side of actin filaments through its NH₂-terminal domain and to the barbed end of filaments through a central domain. The COOH terminus of tensin contains a SH2 (Src homology 2) domain that interacts with tyrosine-phosphorylated proteins and a phosphotyrosine binding domain (PTB) that interacts with the cytoplasmic tail of β -integrins (see Ref. 122 for a review) (Fig. 7D).

The study of tensin knockout mice revealed that this protein plays an important role in kidney and skeletal muscles (87, 124). At the cellular level, the localization of tensin at focal adhesions (123) is mediated by a NH₂-terminal motif distinct from the actin binding site and the PTB domain (37). Tensin also plays a critical role in cell migration. This function depends on both the focal adhesion targeting domains and a functional SH2 domain (36, 37).

Tensin negatively regulates actin assembly by capping the barbed end of filaments through its central domain. This capping activity has been the subject of debates. The first fragment of tensin purified from chicken gizzard called “insertin” was described to inhibit partially barbed end assembly, allowing insertion of monomers between barbed ends and barbed end-bound insertin (191, 246). However, further studies showed that tensin is a true capping protein that completely inhibits actin assembly (39). The capping activity of tensin provides a mechanism to control the transmission of force between the actin cytoskeleton and focal adhesions.

D) α -ACTININ. α -Actinin is an abundant cytoskeletal protein that belongs to the family of structurally related proteins spectrin, dystrophin, and utrophin. This modular protein contains a NH₂-terminal calponin homology (CH)

domain that interacts with actin filaments, central spectrin repeats (SP), and COOH-terminal EF hand motifs (see Refs. 23, 156 for reviews) (Fig. 7D). α -Actinin is a homodimer that cross-links filaments into parallel bundles. The presence of α -actinin along stress fibers and its ability to bundle actin filaments suggest that it plays an important role in controlling the stiffness of these fibers, which may influence the transmission of the contractile force. In focal adhesions, α -actinin interacts with several proteins including the cytoplasmic tail of β -integrins (157), vinculin (234), and zyxin (46). The disruption of the integrin- α -actinin interaction by chromophore-assisted laser inactivation (CALI) induces the retraction of stress fibers, indicating that α -actinin is one of the major links between actin filaments and focal adhesions (173). The VBS may contribute to the activation of vinculin in focal adhesions. Like talin and the *Shigella* protein IpaA, α -actinin binds to vinculin head domain and disrupts the head-to-tail intramolecular interaction (18). Whether α -actinin reveals the barbed end capping function of vinculin has yet to be demonstrated. Interestingly, the VBS of α -actinin is buried in one spectrin repeat located in the rod domain (259). A similar spectrin repeat undergoes dramatic structural changes to form stable intermediates upon mechanical stress (3), suggesting that α -actinin could be a mechanosensitive protein that recruits and activates vinculin in response to actomyosin contraction.

IV. CONCLUSIONS AND PERSPECTIVES

In the last years, the underlying principles that govern actin dynamics associated with membrane protrusion and cell-matrix adhesion during cell migration have started to be unraveled. The development of quantitative cell biology approaches constantly refines our working models. For instance, speckle fluorescence microscopy showed that the observed rates of filament debranching and of capping protein turnover are higher in vivo than the rates measured in vitro, suggesting that in vivo these processes are controlled by mechanisms that are not fully understood. In addition, growing evidence for the contribution of a second actin network, the lamella in membrane protrusion raised new questions, such as the nature of the mechanism that controls the coordination and structural cohesivity between the lamellar and lamellipodial actin meshworks. Since the growth of the lamella is independent of Arp2/3 and ADF, new important concepts in actin nucleation and turnover are expected to emerge from the study of lamella-specific actin binding proteins. Finally, the discovery that membrane curvature proteins contribute to the formation of membrane protrusions changed our simple view that the protrusive force is only generated by actin assembly.

Understanding the regulation of actin assembly in cell-matrix adhesions and its role in the force transmis-

sion to the substrate are even more challenging. Currently, no model can explain how the few known activities are spatiotemporally orchestrated in adhesions. Therefore, the full biochemical characterization of individual actin binding proteins and complexes present in adhesions as well as an in-depth dissection of the correlated motions of these proteins in living cells are required. Despite X-ray structures of individual components and complexes, cryo-EM structures of actin filaments associated with individual components and AFM low-resolution picture of the actin network associated with focal adhesions (61), our understanding of several aspects of adhesion is still limited by the lack of an ultrastructural model of actin filaments barbed end-associated complexes. Cryo-EM studies on whole focal adhesions could fill this gap. The molecular processes underlying the mechanosensitivity of focal adhesion is one of the most exciting questions (for reviews, see Refs. 15, 65). The identity of the deformable proteins that transmit the force of actomyosin contractility to focal adhesions and the mechanism by which these proteins induce the maturation of focal adhesions is still an open issue. Insight into these questions requires the development of conformation-sensitive biosensors of focal adhesions proteins.

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