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# The *Dictyostelium* cytoskeleton

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**Abstract.** New avenues of cytoskeleton research in *Dictyostelium discoideum* have opened up with the cloning of the α- and β-tubulin genes and the characterization of kinesins and cytoplasmic dynein. Much research, however, continues to focus on the actin cytoskeleton and its dynamics during chemotaxis, morphogenesis, and other motile processes. New actin-associated proteins are being identified and characterized by biochemical means and through isolation of mutants lacking individual components. This work is shedding light on the roles of specific actin assemblies in various biological processes.
**Key words.** Actin cytoskeleton; chemotaxis; cytokinesis; microtubules; myosin; phagocytosis.

## Microtubules and associated proteins

Microtubules have many essential roles in eukaryotic cells. They localize and organize organelles, including Golgi and endoplasmic reticulum, and play an important role in intracellular vesicle trafficking. Microtubules also are required for the formation of the mitotic spindle. In contrast to other organisms where the tubulins are products of multi-gene families, *Dictyostelium* α-and β-tubulins are encoded by single genes [120]. Diversity is most likely achieved by post-transcriptional modifications. Kinesin[85] and a cytoplasmic dynein heavy chain[78] have been identified and characterized as microtubule-based motors. These proteins probably support organelle movements along the extensive cytoplasmic microtubule network identified in *D. discoideum*[101,126]. Comparison of the primary sequence of the *Dictyostelium* dynein heavy chain with those from sea urchin flagella suggests the locations of both conserved functional domains and regions specialized for axonemal or cytoplasmic roles[79].

## Actin and actin-related proteins

Actin represents approximately eight percent of all cellular protein in *Dictyostelium*. Seventeen to twenty actin genes are present in the genome, and most are transcribed and translated[99,100]. Although transcription of individual actin genes is developmentally regulated, the resulting proteins are essentially identical in primary sequence[122], suggesting that this multiplicity of genes may regulate actin levels, rather than its interactions with other proteins, at different developemental stages. Diversity of *Dictyostelium* actin function may be conferred through post-translational modifications, which include fatty acid acylation and tyrosine phosphorylation. Actin acylation was first observed in a study of palmitic acid incorporation into membrane proteins[113,114]. Although the role of this modification is unclear, actin is one of the major cellular proteins that covalently incorporates [$^3$H]-palmitic acid during in vivo labeling of the suspension-developed cells. While palmitoylated actin does not cosediment with membranes in distilled water[114], acylation might play a role in the regulation of actin interactions at the membrane or in the cytoplasm under more physiological conditions[113].

Tyrosine phosophorylation of actin has been correlated with shape changes. When *Dictyostelium* amoebae are transferred from starvation buffer to growth media[69,109] or when oxidative phosphorylation is inhibited[73], these cells retract all pseudopods, round up, and become immobile. In both cases, the time course of the cell shape changes correlates with the appearance of a phosphotyrosine eptiope on actin[69,73,109]. In cells lacking the developmentally regulated tyrosine phosphatase PTP1, tyrosine phosphorylation on actin is rapid with a correspondingly accelerated rate of cell rounding[69]. PTP1-minus cells also exhibit a prolonged response, suggesting that PTP1 is involved in tyrosine dephosphorylation. In agreement with this suggestion, overproduction of PTP1 results in decreased tyrosine phosphorylation of actin after transfer from starvation to growth media.

Actin-related proteins (Arps), which are generally 35-55% identical to conventional actin isoforms[108], are also being characterized in *Dictyostelium*. So far, at least two genes encoding members of the Arp2 and Arp3 classes have been identified[2,87]. Although little is known about the proteins encoded by these genes, two are similar to unconventional actins that form a complex with profilin and conventional actin in *Acanthamoeba*[84]. A member of the Arp3 family also encodes a protein that co-localizes and co-purifies with mitochondria[87], an association reminiscent of that observed between G-actin and mitochondria in skeletal muscle[95]. Although not yet

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described, *Dictyostelium* should also contain Arp1, and unconventional actin that facilitates movement of vesicles on microtubules by cytoplasmic dynein and which also implements mitotic spindle orientation and nuclear migration in yeast and filamentous fungi[108].

Motility in *Dictyostelium* is actin-based, and this organism serves as a paradigm for other motile cells[16,28,121]. *Dictyostelium* amoebae exhibit instantaneous velocities as high as 10–15 μm/min[123], placing them among the fastest cells that are also experimentally accessible. They are chemotactically active both in the growth phase and during development. The chemotactic receptors mediating the cAMP response have been identified and their interaction with other components along the signal transduction cascade investigated[63]. Linkages between cAMP binding to its receptor and subsequent cytoskeletal rearrangements are being elucidated through an elegant combination of biochemical and molecular genetic approaches[27]. Rearrangements in the cytoskeleton during chemotactic movements are an area of active research, and several reviews have been published covering this subject[15,17,18,83,106]. Here, it is intended to extend these aspects and summarize data that describe the dynamics in the cytoskeleton during motility and in development.

**The components of the actin cytoskeleton**

Actin in *Dictyostelium* is present at a concentration of 93 (±11) μm[96]. In the resting cell, G- and F-actin are present in approximately equal amounts. In pioneering studies, McRobbie and Newell[86] showed that the actin cytoskeleton undergoes dramatic rearrangements after chemotactic stimulation. Within a few seconds of stimulation of cells with high concentrations of chemoattractant, F-actin increases by 50–60% and becomes incorporated into cytoskeletal structures[53,86]. This peak of actin assembly is rapidly followed by a decrease to almost the initial level by 20 seconds. Then, starting at about 30 seconds post-stimulation, a longer-lasting phase of polymerization occurs that can persist for several minutes. These changes in actin assembly correlate with morphological changes. The depolymerization of F-actin at 20 seconds coincides with a 'cringe' response during which pseudopods are retracted and the cells become completely rounded[49,53], whereas the second increase in actin content parallels the extension of new pseudopods.

**Regulators of actin assembly and disassembly**

Actin-associated proteins involved in the regulation of such complicated responses have been isolated from many organisms. These proteins can be divided into two major classes, based on whether their primary interactions are with G- or F-actin.

In *Dictyostelium*, the major proteins responsible for sequestering monomeric (G)-actin appear to be profilin I and profilin II. These proteins share 68 out of 126 amino acids (55% identity) and are 62% identical at the DNA level[58]. While both profilins reduce the rate and extent of actin polymerization in vitro, profilin II, which has a slightly higher affinity for G-actin ($K_D$ of ~ 1.8 μM vs. ~ 5.1 μM), may be somewhat more effective. The resulting inhibition of spontaneous nucleation of new actin filaments may be the chief intracellular function of profilin. However, other roles for profilin during actin polymerization also are possible[50,94,117]. In addition to its G-actin binding activity, profilin has been shown to bind $PIP_2$, to potentiate ADP/ATP exchange on actin monomers, and to promote the efficient addition of ATP-actin to the fast-assembling ends of filaments. *Dictyostelium* mutants lacking both profilins contain proportionately less unpolymerized cytoplasmic actin and proportionately more F-actin, demonstrating a role for profilin in maintaining the normal ratio of G- to F-actin[59]. Loss of the profilins also resulted in altered growth in shaking suspension, decreased rates of motility, and defects in cytokinesis and development. These phenotypic defects are likely consequences of the altered ratio of G- to F-actin and the reduced amounts of available G-actin. Interestingly, the profilin double mutants still contained about 55 μm unpolymerized actin, suggesting the presence of yet-undetermined additional actin-sequestering proteins. Functional analogues of β-thymosins, which sequester actin monomers in sea urchins and vertebrates[88], are obvious candidates for this additional sequestering activity, but such proteins have not yet been described in *Dictyostelium*[59].

As other amoeboid cells[115], *Dictyostelium* contains a large number of F-actin binding proteins that control the localization, length, and stability of actin filaments. Due to the presence of monomer-sequestering proteins, localization of actin assembly in the cell is controlled by proteins that regulate elongation at the fast-growing "plus" ends of actin filaments. Such localized actin assembly is required for pseudopod formation during chemotaxis and for regulation of other three-dimensional actin based structures, such as filopodia and membrane ruffles.

Sites of actin filament assembly in the cell are regulated by proteins that promote the nucleation of new actin filaments and by proteins that control the number of accessible fast-growing "plus" ends on existing filaments through regulated capping and/or filament severing activities. The major membrane-associated actin nucleating protein in *Dictyostelium* appears to be ponticulin, a protein of unusual structure that spans the plasma membrane[64,129]. Ponticulin binds to the sides of actin filaments and nucleates actin assembly by potentiating the formation of trimeric actin nuclei that are free to elongate from both ends[12,110]. Deletion of ponticulin from the plasma membrane by immunological and genetic methods leads to a loss of membrane-mediated

actin binding and nucleation, demonstrating that ponticulin is a major high affinity link between the membrane and the actin cortex in *Dictyostelium*[65,110]. The presence of an immunologically crossreactive protein of similar size in plasma membranes from polymorphonuclear leukocytes suggests the existence of a mammalian analog[130].

Hisactophilin is a peripheral membrane protein that binds actin and mediates actin nucleation, at least in low ionic strength solutions[104]. First identified in a gel overlay assay with radioactively labeled actin monomers[105], hisactophilin is a protein with 31 surface exposed histidines that also binds F-actin at pH values below 7.5[52,104]. Each of the two isoforms of hisactophilin is myristoylated and capable of associating with the plasma membrane[55], where histactophilin may function as a pH-sensitive actin binding protein. Association with the membrane also may be regulated by phosphorylation and/or formation of homo- and heterodimers of the two isoforms[55].

*Dictyostelium* also contains a homologue of talin[80], a cytoplasmic protein that in mammalian cells binds actin, nucleates actin assembly at high concentrations, and localizes to sites of actin attachment to substratum[4]. *Dictyostelium* talin, previously called filopodin, is 24% identical to mouse talin overall and contains amino- and carboxy-terminal domains that are 44% and 52% identical, respectively, to the corresponding sequences in the mouse protein[80]. In *Dictyostelium*, talin exhibits a diffuse cytoplasmic localization with concentrations at the tips of filopodia and at the leading edges of polarized cells.

The rapid polymerization of F-actin following a chemotactic stimulus has been proposed to result from the uncapping of many short actin filaments[9,54]. In this model, growth of filaments in the unstimulated cell is inhibited by capping proteins that are bound to the fast-growing ends of filaments; chemoattractants act by inducing the release of the cap, permitting rapid elongation to occur. The agonist-regulated activity responsible for inhibiting actin elongation in the resting cell has been named aginactin[103]. Aginactin activity is associated with Hsc70[33], a heat shock cognate protein involved in proper folding of proteins. Interestingly, Hsc70, which contains an aminoterminal ATPase domain with structural similarity to the actin monomer[42], co-purifies with *Dictyostelium* cap32/34[61], a highly conserved heterodimeric capping protein that binds to the fast-growing ends of actin filaments and efficiently inhibits filament elongation[56,57]. Hsc70 and cap32/34 co-sediment after immunoprecipitation with antibodies against either subunit of the capping protein, suggesting the existence of a ternary complex. Hsc70 not only promotes the correct folding of cap32/34, but the actin-like amino-terminal domain of Hsc70 also appears to directly stimulate cap32/34 activity[61]. Like its mammalian homologue[62], cap32/34 also may be regulated by anionic phospholipids since preincubation of cap32/34 with phosphatidylinositol 4,5-bisphosphate ($PIP_2$) inhibits its actin binding and capping activities in vitro[60].

Cap100 (protovillin) is a second capping protein found in *Dictyostelium*. Cap100 also binds to and blocks the fast-growing ends of actin filaments in a $PIP_2$-inhibitable manner[67]. Conceptual translation of the cap100 coding sequence reveals that this protein is very similar to villin[68], a vertebrate protein that bundles actin filaments in the microvilli of epithelial cells and, in the presence of $Ca^{2+}$, severs actin filaments[43]. Cap100 lacks the $Ca^{2+}$-senstivity and F-actin bundling, nucleating, and severing activities of villin[67], probably due to the absence of 8 amino acids from a region that, in villin, mediates F-actin binding in the presence of $Ca^{2+}$ [44]. Because it appears that a capping protein like cap100 was the evolutionary precursor of vertebrate villin, this protein has been renamed "protovillin"[68].

Reorganization of actin-based cytoskeletal structures often requires the fragmentation of existing filaments and filament bundles. In *Dictyostelium*, the principal severing protein is called severin[8,131]. In the presence of micromolar $Ca^{2+}$, severin cuts actin filaments, binds tightly to the fast-growing filament ends, and nucleates actin assembly. Actin binding, capping, and severing activities are inhibited by $PIP_2$ and other negatively charged phospholipids[36,133]. Structurally, severin resembles other calcium-activated actin-severing proteins, such as fragmin from *Physarum* and gelsolin and villin from higher organisms[1]. These severing proteins are all built from three (severin, fragmin) or six (gelsolin, villin) highly homologous domains. In severin, the first domain is responsible for the capping activity, whereas domains 2 and 3 contain binding sites for the sides of actin filaments[35,36]. Domain 2 of severin, which contains a $Ca^{2+}$-dependent actin-binding site, exhibits a three dimesional structure similar to that of profilin and domain I of gelsolin[97,107]. These similar structures are stabilized by highly conserved residues.

Three-dimensional cortical networks are stabilized by actin crosslinking and bundling proteins. In *Dictyostelium*, these stabilizing proteins include the highly conserved proteins, filamin (ABP-240), gelation factor (ABP-120), α-actinin, fodrin (ABP-220, spectrin), EF-1a (ABP-50), p30a, and comitin (reviewed in refs 17, 18, 83, 106). ABP-240 and ABP-120 (gelation factor) are, as the names imply, actin binding proteins with apparent sizes of 240,000 and 120,000, respectively. Both are dimeric actin crosslinking proteins with structural similarites to vertebrate filamin[14,66,89]. These similarities include an actin-binding consensus sequence near the aminoterminus[6] that is also found in other, more distant members of the spectrin superfamily, including the dimeric actin bundling protein α-actinin and the mammalian actin-membrane linking proteins, spectrin and dystrophin[32].

Elongation factor-1a (EF-1a) was isolated from *Dictyostelium* as a 50 kDa actin filament-binding and bundling protein that is localized in filopodia extended in response to chemoattractant[26,29,132]. Besides its well-recognized role in protein biosynthesis, EF-1a also may affect cortical structure by organizing actin filaments into close-packed bundles that exclude other actin binding proteins[93] and/or through a recently described microtubules-severing activity[111].

The 30 kDa actin-bundling protein (p30a) is a highly conserved filopodial protein that crosslinks actin filaments in the presence of low, but not high (micromolar), concentrations of $Ca^{2+}$ [38,47,70]. This protein is unique in that it also inhibits disassembly from both ends of the actin filament without blocking filament elongation or affecting the critical concentration of actin monomers needed for assembly[135]. Thus, p30a may both bundle actin filaments and protect them from disassembly, thereby promoting the formation of localized filament arrays with enhanced stability. The deduced sequence of p30a is novel but contains two EF-hands that are probably responsible for the protein's $Ca^{2+}$-sensitivity and a short segment that is homologous to sequences in ezrin and caldesmon[39]. Based on its concentration in filopodia and at sites of cell-cell association[40], p30a is thought to participate in the bundling and stabilization of the filaments at these sites.

Comitin (p24), a 24-kDa G- and F-actin binding protein isolated by Stratford and Brown[116], is a filament-crosslinking protein associated Golgi and vesicle membranes[90,124]. The basis for comitin binding to membranes is not yet characterized. Comitin contains a series of carboxy-terminal glycine-tyrosine-proline repeats similar to those found at the amino-terminus of annexin VII, a conserved protein implicated in membrane fusion events required for secretion in *Dictyostelium* and vertebrates[31].

Most of the actin-binding proteins described above are structurally and/or functionally similar to cognate proteins described in vertebrates. F-actin binding proteins that, so far, are unique to *Dictyostelium* include hisactophilin, coronin, coactosin, and p30b. Both coronin and coactosin have been isolated from actomyosin complexes[23,24]. Localized in cell surface projections, coronin is a 49 kDa protein that contains a WD40-motif found in other proteins, including the $\beta$-subunits of heterotrimeric G-proteins[23]. Coactosin is a 17 kDa protein that exhibits structural similarities (24–26% identity) with vertebrate cofilins and with sequences in mammalian drebrin and yeast ABPlp, actin-binding proteins involved in cell shape regulation[24]. P30b is a $Ca^{2+}$-insensitive actin-bundling protein that is diffusely distributed in the cytoplasm[7,91].

**Myosins**

*Dictyostelium* contains 10 to 13 myosin heavy chain genes identified by molecular cloning and physical mapping[72,119]. Of these, one is a conventional non-muscle myosin II that oligomerizes into bipolar filaments during localized cortical contractions involved in cytokinesis, morphogenesis, and capping of cell surface receptors (reviewed in ref. 112). The assembly of myosin II into filaments is inhibited by phosphorylation of three threonines in the carboxy-terminal tail of the protein[34,81]. Conversely, phosphorylation of the regulatory 18 kDa light chain stimulates the actin-activated ATPase and motility activities of myosin II in vitro[51]. However, light phosphorylation is apparently not required for myosin II function in vivo[92]. At least five of the myosin heavy chains known from DNA sequencing are myosin Is. These myosins are single-headed, nonfilamentous proteins with a highly conserved amino-terminal motor domain containing the actin-activated ATPase but with distinct carboxy-terminal tails. Consensus motifs, called tail homology domains, are responsible for binding to membranes, for an ATP-independent actin-binding site, and/or for binding to proline-rich sequences in other proteins. Individual myosin isoforms have been immunolocalized to extending pseudopods[45,72]. However, mutant amoebae lacking either myosin IA or myosin IB form many more pseudopodia directed away from the source of a chemoattractant and exhibit increased frequencies of turning[118,125], suggesting that these proteins normally play a role in the repression of pseudopod extension. Antibodies that inhibit the in vitro activity of myosin IC, an isoform concentrated at the periphery of the contractile vacuole[134], block the function of this organelle in living cells[30]. Thus, the myosins Is apparently mediate cortical structure, contractile or signaling processes at the plasma membrane, and/or vesicle motility. While some of the uncharacterized myosin heavy chain genes may encode additional myosin I isoforms, it is likely that some represent members of the five other known classes of unconventional myosins[11].

**Dynamics of actin-binding proteins during chemotaxis**

Many cytoskeletal components are abundant and localized throughout the cytoplasm without obvious enrichment in specific structures or regions of the cell. For instance, immunolocalization of severin, $\alpha$-actinin, or gelation factor reveals a diffuse cytoplasmic distribution. However, many of these proteins become relocated in response to extracellular stimuli or during development. Chemotactic stimulation, for example, leads to an enrichment of the gelation factor in extending pseudopods, an observation that has been suggested to indicate the involvement of this protein in pseudopod extension following stabilization of the three-dimensional actin network[19]. Similarly, the bundling protein

p30a associates with the actin cytoskeleton within 5 to 10 seconds after a chemotactic stimulus[91], talin relocates to the front of the cell within about 30 seconds[80], and the bundling protein ABP50 incorporates into filopodia by 90 seconds post-stimulation[29]. Myosin II association with the cytoskeleton increases at about 20 seconds after stimulation during the 'cringe', and then disassociates before directional pseudopod extension at 60 to 90 seconds post stimulation[29].

**Actin and actin-binding proteins in phagocytosis**

The actin-based cytoskeleton plays a major role during the initial stages of phagoctyosis, which is the engulfment of particles by cells. Actin filaments are recruited to the cytoplasmic membrane surface at the site of attachment[5], and cytochalasins, which disassemble actin filaments, block particle internalization[3]. The recruitment of actin to the membrane surface is defective in recently described phagocytosis mutants generated by chemical mutagenesis of *Dictyostelium*[13]. These cell lines, which grow in liquid culture but not when cultured on bacteria, bind normally to bacteria but do not exhibit the localized polymerization of actin filaments required for ingestion.

The bundling protein p30a may be involved in actin-mediated particle internalization because of a monoclonal antibody that blocks p30a binding to F-actin in vitro decreases phagocytosis after introduction into *Dictyostelium* by controlled sonication[46]. Localized around the food particles during the initial stages of engulfement, p30a dissociates from the phagosome before internalization is complete. By constrast, α-actinin is initially recruited to the phagocytic cup later in the process but remains associated with the maturing phagosome after p30a has disassociated[48]. These studies demonstrate spatial and temporal differences in the actin assemblies that participate in phagocytosis.

*Dictyostelium* mutants that lack individual, well-characterized actin-binding proteins have, so far, not indicated a strict dependence of phagocytosis on any of these particular proteins. However, myosin I mutants exhibit slight defects in phagocytosis[71], and mutants lacking both profilin isoforms show a significant growth reduction when cultured on a lawn of bacteria[59]. On the other hand, uptake of latex beads in suspension is not impaired in the profilin double mutants.

**Actin-binding proteins in cytokinesis**

The cleavage furrow is another cytoskeletal structure that is accessible to analysis by immunological techniques. During cytokinesis, cleavage of the cell occurs by the contraction of actin and myosin II filaments at the cell equator[41,102]. The resulting cleavage furrow is a transient structure with an array of actin filaments that appear to be attached at or near their fast-polymerizing ends to plaques associated with the plasma membrane. The interaction of these actin filaments with myosin II filaments applies tension to the membrane. Myosin II relocation during cytokinesis is highly dynamic in *Dictyostelium*[75]. Mutants lacking the myosin II heavy chain cannot undergo cytokinesis in suspension culture[25,76], confirming previous evidence that myosin II is the motor for the process[102]. An identical phenotype is observed upon inactivation of either the myosin II regulatory or essential light chain genes[10,92,98]. Like the heavy chain mutants, these cells fail to form cleavage furrows and thus become highly multinucleated in suspension. However, on solid substrates, all three types of myosin II mutants are able to divide into daughter cells by a mechanism termed 'traction-mediated cytofission'. Other actin-associated proteins that appear to play a role in cytokinesis include p30a, profilin, and coronin. The involvement of p30a is suggested by its localization to the cleavage furrow in immunofluorescence microscopy[48]. Roles for profilin and coronin have been demonstrated by mutant analyses[24,59]. Although profilin is not obviously concentrated in cleavage furrows, profilin minus double mutants are unable to divide in shaking suspension and form large multinucleated cells[59]. This defect is overcome by attachment to a solid substrate, implying that the cells probably divide by traction-mediated cytofission, as described above for myosin II-minus cells. Thus, it appears that regulation of the G- and F-actin ratio, or another function of profilin[50,94,117], is required for normal cytokinesis. *Dictyostelium* mutants lacking coronin also are defective for cytokinesis in liquid suspension[24]. Since this protein is localized in distal regions of the dividing cell and is, in fact, depleted from the cleavage furrow[23,24], an involvement in generating traction forces can be envisioned.

**Actin-binding proteins in development**

In general, message levels of the actin-binding proteins analyzed so far are not strongly regulated during development, although many of these proteins increase in amount during early development[127]. Regulation of the ponticulin transcript is unique in this respect because the transcripts decrease dramatically after aggregation, whereas the protein remains present albeit at lower levels[64]. Alterations in the localizations of actin-binding proteins during cell shape changes and development also have been observed. For instance, the bundling protein p30a is enriched at sites of cell-cell contact during streaming and early aggregation[40,91].

**Insights from mutants**

Insights from *Dictyostelium* mutants lacking one or more cytoskeletal proteins have contributed significantly to our understanding of the cytoskeleton in living cells. One of the great strengths of *Dictyostelium* as an experimental system is the relative ease with which mutants defective in motile functions can be generated and analyzed[74,82]. Among the most obvious phenotypes of these mutants are defects in cell patterning and

multicellular development. For instance, cells lacking ponticulin aggregate faster than wild-type cells but become highly asynchronous during later development, although viable spores eventually form[65]. Loss of the myosin II heavy chain or either myosin II light chain leads to a developmental block after aggregation[10,77]. Mutants lacking the myosin II heavy chain show chemotaxis and form two-dimensionsal aggregation streams. However, cell movement is chaotic, and the ensuing loose aggregates lack the morphological 'tips' found on mounds of wild-type cells. The failure of myosin II mutants to develop beyond the mound stage may be caused by the loss of intracellular forces needed for coordinated movement and/or by defective chemotactic signaling within the cell mass[37]. Development is also impaired in profilin double mutants and in mutants lacking two actin filament crosslinking proteins ($\alpha$-actinin and the gelation factor)[59,128]. These mutants differentiate into prestalk and prespore cells, but no further morphogenesis occurs. In many cases, mutant cells complete development when mixed with wild-type cells. In such co-aggregation studies, the profilin mutants and the crosslinkers double mutants preferentially develop into spores. This observation may indicate that a highly dynamic cytoskeleton is required for the motile activities of prestalk cells.

Further advances in understanding the role of the cytoskeleton in cell movement and development will require identification of the biochemical pathways through which extracellular signals induce cytoskeletal changes. These analyses will be aided by defined conditions that permit the experimental isolation of specific signaling events[73]. Also, new proteins will arise from the combination of conventional biochemical techniques and mutant analyses that is almost uniquely possible in *Dictyostelium*. Furthermore, the availability of physical maps and YAC libraries representing most of the *Dictyostelium* genome permit the identification of novel members of multigene families[119]. These technical advantages facilitate the identification, cloning, and functional characterization of a wide range of cytoskeletal proteins involved in diverse motile processes.


1 André, E., Lottspeich, F., Schleicher, M., and Noegel, A., Severin, gelsolin, and villin share a homologous sequence in regions presumed to contain F-actin severing domains. J. biol. Chem. *263* (1988) 722–727.

2 Atkinson, S. J., and Pollard, T. D., Profilin-associated unconventional actins from *Dictyostelium discoideum*. Molec. Biol. Cell *5* (1994) 157a.

3 Axline, S. G., and Reaven, E. P., Inhibition of phagocytosis and plasma membrane mobility of the cultivated macrophage by cytochalasin B. J. Cell Biol. *62* (1974) 647–659.

4 Beckerle, M. C., and Yeh, R. K., Talin: Role at sites of cell-substratum adhesion. Cell Motil. Cytoskel. *16* (1990) 7–13.

5 Boyles, J., and Bainton, D. F., Changes in plasma-membrane-associated filaments during endocytosis and exocytosis in polymorphonuclear leukocytes. Cell *24* (1981) 905–914.

6 Bresnick, A. R., Janmey, P. A., and Condeelis, J., Evidence that a 27-residue sequence is the actin binding site of ABP-120. J. biol. Chem. *266* (1991) 12989–12993.

7 Brown, S. S., A $Ca^{2+}$ insensitive actin-crosslinking protein from *Dictyostelium discoideum*. Cell Motil. *5* (1985)529–543.

8 Brown, S. S., Yamamoto, K., and Spudich, J. A., A 40,000-dalton protein from *Dictyostelium discoideum* affects assembly of actin in a $Ca^{2+}$-dependent manner. J. Cell Biol. *93* (1982) 205–210.

9 Cano, M. L., Lauffenburger, D. A., and Zigmond, S. H., Kinetic analysis of F-actin depolymerization in polymorphonuclear leukocyte indicates that chemoattractant stimulation increases actin filament number without altering the filament length distribution. J. Cell Biol. *115* (1991) 677–687.

10 Chen, P., Ostrow, B. D., Tafuri, S. R., and Chisholm, R. L., Targeted disruption of the *Dictyostelium* RMLC gene produces cells defective in cytokinesis and development. J. Cell. Biol. *127* (1994) 1933–1944.

11 Cheney, R. E., Riley, M. A., and Mooseker, M. S., Phylogenetic analysis of the myosin superfamily. Cell Motil. Cytoskel. *24* (1993) 215–223.

12 Chia, C. P., Shariff, A., Savage, S. A., and Luna, E. J., The integral membrane protein, ponticulin, acts as a monomer in nucleating actin assembly. J. Cell Biol. *120* (1993) 909–922.

13 Cohen, C. J., Bacon, R., Clarke, M., Joiner, M., and Mellman, I., *Dictyostelium discoideum* mutants with conditional defects in phagocytosis. J. Cell Biol. *126* (1994) 955–966.

14 Condeelis, J., Salisbury, J., and Fujiwara, K., A new protein that gels F-actin in the cell cortex of *Dictyostelium discoideum*. Nature Lond. *292* (1981) 161–163.

15 Condeelis, J., Are all pseudopods created equal? Cell Motil. Cytoskel. *22* (1992) 1–6.

16 Condeelis, J., Jones J. and Segall, J. E., Chemotaxis of metastatic tumor cells: Clues to mechanisms from the *Dictyostelium* paradigm. Cancer and Metastasis Rev. *11* (1992) 55–68.

17 Condeelis, J., Understanding the cortex of crawling cells: insights from *Dictyostelium*. Trends Cell Biol. *3* (1993) 371–376.

18 Condeelis, J., Life at the leading edge: the formation of cell protrusions. A. Rev. Cell Biol. *9* (1993) 411–444.

19 Condeelis, J., Bresnick, A., Demma, Dharmawardhane, S., Eddy, R., Hall, A., Sauterer, R., and Warren, V., Mechanisms of amoeboid chemotaxis: an evaluation of the cortical expansion model. Devl Genet. *11* (1990) 333–340.

20 Cox, D., Condeelis, J., Wessels, D., Soll, D., Kern, H., and Knecht, D., Targeted disruption of the ABP-120 gene leads to cells with altered motility. J. Cell Biol. *116* (1992) 943–955.

21 Cox, D., Ridsdale, J. A., Condeelis, J., and Hartwig, J., Genetic deletion of ABP-120 alters the three-dimensional organization of actin filaments in *Dictyostelium* pseudopods. J. Cell Biol. *128* (1995) 819–835.

22 de Hostos, E. L., Bradtke, B., Lottspeich, F., and Gerisch, G., Coactosin, a 17 kDa F-actin binding protein from *Dictyostelium discoideum*. Cell Motil. Cytoskel. *26* (1993) 181–191.

23 de Hostos, E. L., Bradtke, B., Lottspeich, F., Guggenheim, G, and Gerisch, G., Coronin, an actin binding protein of *Dictyostelium discoideum* localized to cell surface projections, has sequence similarities to G protein $\beta$ subunits. EMBO J. *10* (1991) 4097–4104.

24 de Hostos, E. L., Rehfueß, C., Bradtke, B., Waddell, D. R., Albrecht, R., Murphy, J., and Gerisch, G., *Dictyostelium* mutants lacking the cytoskeletal protein coronin are defective in cytokinesis and cell motility. J. Cell Biol. *120* (1993) 163–173.

25 De Lozanne, A., and Spudich, J. A., Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination. Science *236* (1987) 1086–1091.

26 Demma, M., Warren, V., Hock, R., Dharmawardhane, S., and Condeelis, J., Isolation of an abundant 50,000-dalton actin filament bundling protein from *Dictyostelium* amoebae, J. biol. Chem. *265* (1990) 2286–2291.

27 Devreotes, P. N., G protein-linked signaling pathways control the developmental program of *Dictyostelium*. Neuron *12* (1994) 235–241.

28 Devreotes, P. N., and Zigmond, S., H., Chemotaxis in eukaryotis cells: a focus on leukocytes and *Dictyostelium*. A. Rev. Cell Biol. *4* (1988) 649–686.

29 Dharmawardhane, S., Demma, M., Yang, F., and Condeelis, J. Compartmentalization and actin binding properties of ABP-50: The elongation factor-1 alpha of *Dictyostelium*. Cell Motil. Cytoskel. *20* (1991) 279–288.

30 Doberstein, S. K., Baines, I. C., Wiegand, G., Korn, E. D., and Pollard, T. D., Inhibition of contractile vacuole function in vivo by antibodies against myosin-I. Nature, Lond. *365* (1993) 841–843.

31 Döring, V., Schleicher, M., and Noegel, A. A., *Dictyostelium* annexin VII (synexin). J. biol. Chem. *266* (1991) 17509–17515.

32 Dubreuil, R. R., Structure and evolution of the actin crosslinking proteins. BioEssays *13* (1991) 219–226.

33 Eddy, R., Sauterer, R., and Condeelis, J., Aginactin, an agonist-regulated F-actin capping activity is associated with an HSP70 in *Dictyostelium*. J. biol. Chem. *268* (1993) 23267–23274.

34 Egelhoff, T. T., Lee, R. J., and Spudich, J. A., *Dictyostelium* myosin heavy chain phosphorylation sites regulate myosin filament assembly and localization in vivo. Cell *75* (1993) 363–371.

35 Eichinger, L., Noegel., A. A., and Schleicher, M., Domain structure in actin binding proteins: expression and functional characterization of truncated severin. J. Cell Biol. *112* (1991) 665–676.

36 Eichinger, L., and Schleicher, M., Characterization of actin- and lipid-binding domains in severin, a $Ca^{2+}$-dependent F-actin fragmenting protein. Biochemistry *31* (1992) 4779–4787.

37 Eliot, S., Joss, G. H., Spudich, A., and Williams, K. L., Patterns in *Dictyostelum discoideum*: the role of myosin II in the transition from the unicellular to the multicellular phase. J. Cell Sci. *104* (1993) 457–466.

38 Fechheimer, M., The *Dictyostelium discoideum* 30,000-dalton protein is an actin filament-bundling protein that is selectively present in filopodia. J. Cell. Biol. *104* (1987) 1539–1551.

39 Fechheimer, M., Murdock, D., Carney, M., and Glover, C. V. C., Isolation and sequencing of cDNA clones encoding the *Dictyostelium discoideum* 30,000-dalton actin-bundling protein. J. biol. Chem. *266* (1991) 2883–2889.

40 Fechheimer, M., Ingalls, H. M., Furukawa, R., and Luna, E. J., Association of the *Dictyostelium* 30 kDa actin bundling protein with contact regions. J. Cell Sci. *107* (1994) 2393–2401.

41 Fishkind, D. J., and Wang, Y.-L., Orientation and three-dimensional organization of actin filaments in dividing cultured cells. J. Cell Biol. *123* (1993) 837-848.

42 Flaherty, K. M., Deluca-Flaherty, C., and McKay, D. B., Three-dimensional structure of the ATPase fragment of a 70 K heat-shock cognate protein. Nature, Lond. *346* (1990) 623–628.

43 Friederich, E., Pringault, E., Arpin, M., and Louvard, D., From the structure to the function of villin, an actin-binding protein of the brush border. BioEssays *12* (1990) 403–408.

44 Friedrich, E., Vancompernolle, K., Huet, C., Goethals, M., Findori, J., Vanderkerckhove, J., and Louvard, D., An actin-binding site containing a conserved motif of charged amino acid residues is essential for the morphogenetic effect of villin. Cell *70* (1992) 81–92.

45 Fukui, Y., Lynch, T. J., Brzeska, H., and Korn, E. D., Mysoin I is located at the leading edges of locomoting *Dictyostelium* amoebae. Nature, Lond. *341* (1989) 328-331.

46 Furukawa, R., Butz, S., Fleischmann, E., and Fechheimer, M., The *Dictyostelium discoideum* 30,000 dalton protein contributes to phagocytosis. Protoplasma *169* (1992) 18–27.

47 Furukawa, R., and Fechheimer, M., Localization, expression, evolutionary conservation, and structure of the 30,000 dalton actin bundling protein of *Dictyostelium discoideum*. Devl Genet. *11* (1990) 362–368.

48 Furukawa, R., and Fechheimer, M., Differential localization of α-actinin and the 30 kD actin-bundling protein in the cleavage furrow, phagocytic cup, and contracile vacuole of *Dictyostelium discoideum*. Cell Motil. Cytoskel. *29* (1994) 46–56.

49 Futrelle, R. P., Traut, J., and McKee, W. G., Cell behaviour in *Dictyostelium* preaggregation response to localized cyclic AMP pulses. J. Cell Biol. *92* (1982) 807–821.

50 Goldschmidt-Clermont, P. J., and Janmey, P. A., Profilin, a weak CAP for actin and RAS. Cell *66* (1991) 419–421.

51 Griffith, L. M., Downs, S. M., and Spudich, J. A., Myosin light chain kinase and myosin light chain phosphatase from *Dictyostelium*: Effects of reversible phosphorylation on myosin structure and function. J. Cell Biol. *104* (1987) 1309–1323.

52 Habazettl, J., Gondol, D., Wiltscheck, R., Otlewski, J., Schleicher, M., and Holak, T. A., Structure of hiscatophilin is similar to interleukin-1β and fibroblast growth factor. Nature, Lond. *359* (1992) 855–858.

53 Hall, A., Schlein, A., and Condeelis, J., Relationship of pseudopod extension to chemotactic hormone-induced actin polymerization in amoeboid cells. J. Cell Biochem. *37* (1988) 285–299.

54 Hall, A. L., Warren, V., Dharmawardhane, S., and Condeelis, J., Identification of actin nucleation activity and polymerization inhibitor in ameboid cells: Their regulation by chemotactic stimulation. J. Cell Biol. *109* (1989) 2207–2213.

55 Hanakam, F., Eckerskorn, C., Lottspeich, F., Müller-Taubenberger, A., Schäfer, W., and Gerisch, G. The pH-sensitive actin-binding protein hisactophilin of *Dictyostelium* exists in two isoforms which both are myristoylated and distributed between plasma membrane and cytoplasm. J. biol. Chem. *270* (1995) 596–602.

56 Hartmann, H., Noegel, A. A., Eckerskorn, C., Rapp, S., and Schleicher, M., $Ca^{2+}$-independent F-actin capping proteins. J. biol. Chem. *264* (1989) 12639–12647.

57 Hartmann, H., Schleicher, M., and Noegel, A. A., Heterodimeric capping proteins constitute a highly conserved group of actin-binding proteins. Devl Genet. *11* (1990) 369–376.

58 Haugwitz, M., Noegel, A. A., Rieger, D., Lottspeich, F., and Schleicher, M., *Dictyostelium discoideum* contains two profilin isoforms that differ in structure and function. J. Cell Sci. *100* (1991) 481–489.

59 Haugwitz, M., Noegel, A. A., Karakesisoglou, J., and Schleicher, M., *Dictyostelium* amoebae that lack G-actin-sequestering profilins show defects in F-actin content, cytokinesis, and development. Cell *79* (1994) 303–314.

60 Haus, U., Hartmann, H., Trommler, P., Noegel, A. A., and Schleicher, M., F-actin capping by cap32/34 requires heterodimeric conformation and can be inhibited with $PIP_2$. Biochem. biophys. Res. Commun. *181* (1991) 833–836.

61 Haus, U., Trommler, P., Fisher, P. R., Hartmann, H., Lottspeich, F., Noegel, A. A., and Schleicher, M., The heat shock cognate protein from *Dictyostelium* affects actin polymerization through interaction with the actin-binding protein cap32/34. EMBO J. *12* (1993) 3763–3771.

62 Heiss, S. G., and Cooper, J. A., Regulation of capZ, an actin capping protein of chicken muscle, by anionic phospholipids. Biochemistry *30* (1991) 8753–8758.

63 Hereld, D., and Devreotes, P. N., The cAMP receptor family of *Dictyostelium*. Int. Rev. Cytol. *137B* (1992) 35–47.

64 Hitt, A. L., Lu, T. H., and Luna, E. J., Ponticulin is an atypical membrane protein. J. Cell Biol. *126* (1994) 1421–1431.

65 Hitt, A., L., Hartwig, J. H., and Luna, E., J., Ponticulin is the major high affinity link between the plasma membrane and the cortical actin network in *Dictyostelium*. J. Cell Biol. *126* (1994) 1433–1444.

66 Hock, R. S., and Condeelis, J. S., Isolation of a 240-kilodalton actin-binding protein from *Dictyostelium discoideum* J. biol. Chem. *262* (1987) 394–400.

67 Hofmann, A., Eichinger, L., André, E., Rieger, D., and Schleicher, M., Cap 100, a novel phosphatidylinositol-4, 5-bisphosphate-regulated protein that caps actin filaments but does not nucleate actin assembly. Cell Motil. Cytoskel. *23* (1992) 133–144.

68 Hofmann, A., Noegel, A. A., Bomblies, L., Lottspeich, F., and Schleicher, M., The 100 kDa F-actin capping protein of *Dictyostelium* amoebae is a villin prototype (protovillin). FEBS Lett. *328* (1993) 71–76.

69 Howard, P. K., Sefton, B. M., and Firtel, R. A., Tyrosine phosphorylation of actin in *Dictyostelium* associated with cell-shape changes. Science *259* (1993) 241–244.

70 Johns, J. A., Brock, A. M., and Pardee, J. D., Colocalization of F-actin and 34-kilodalton actin bundling protein in *Dictyostelium* amoebae and cultured fibroblasts. Cell Motil. Cytoskel. *9* (1988) 205–218.

71 Jung, G., and Hammer, J. A., Generation and characterization of *Dictyostelium* cells deficient in a myosin heavy chain isoform. J. Cell Biol. *110* (1990) 1955–1964.

72 Jung, G., Fukui, Y., Martin, B., and Hammer III, J. A., Sequence, expression pattern, intracellular localization, and targeted disruption of the *Dictyostelium* myosin ID heavy chain isoform. J. biol. Chem. *268* (1993) 14981–14990.

73 Jungbluth, A., von Arnim, V., Biegelmann, E., Humbel, B., Schweiger, A., and Gerisch, G., Strong increase in the tyrosine phosphorylation of actin upon inhibition of oxidative phosphorylation: correlation with reversible rearrangements in the actin skeleton of *Dictyostelium* cells. J. Cell Sci. *107* (1994) 117–125.

74 Kessin, R. H., Genetics of early *Dictyostelium discoideum* development. Microbiol. Rev. *52* (1988) 29–49.

75 Kitanishi-Yumura, T., and Fukui, Y., Actomyosin organization during cytokinesis: reversible translocation and differential redistribution in *Dictyostelium*. Cell Motil. Cytoskel. *12* (1989) 78–89.

76 Knecht, D. A., and Loomis, W. F., Antisense RNA inactivation of myosin heavy chain gene expression in *Dictyostelium discoideum*. Science *236* (1987) 1081–1086.

77 Knecht, D. A., and Loomis, W. F., Developmental consequences of the lack of myosin heavy chain in *Dictyostelium discoideum*. Devl Biol. *128* (1988) 178–184.

78 Koonce, M. P., and McIntosh, J. R., Identification and immunolocalization of cytoplasmic dynein in *Dictyostelium*. Cell Motil. Cytoskel. *15* (1990) 51–62.

79 Koonce, M. P., Grissom, P. M., and McIntosh, J. R., Dynein from *Dictyostelium*: Primary structure comparisons between a cytoplasmic motor enzyme and flagellar dynein. J. Cell Biol. *119* (1992) 1598–1604.

80 Kreitmeier, M., Gerisch, G., Heizer, C., and Müller-Taubenberger, A., A talin homologue of *Dictyostelium* rapidly assembles at the leading edge of cells in response to chemoattractant. J. Cell Biol. *129* (1995) 179–188.

81 Kuczmarski, E. R., and Spudich, J. A., Regulation of myosin self-assembly: Phosphorylation of *Dictyostelium* heavy chain inhibits formation of thick filaments. Proc. natl Acad. Sci. USA *77* (1980) 7292–7296.

82 Kuspa, A., and Loomis, W. F., Transformation of *Dictyostelium*: Gene disruptions, insertional mutagenesis, and promoter traps. Methods Molec. Genet. *3* (1994) 3–21.

83 Luna, E., J., and Condeelis, J., Actin associated proteins in *Dictyostelium discoideum*. Devl Genet. *11* (1990) 328–332.

84 Mackesky, L. M., Atkinson, S. J., Ampe, C., Vandekerckhove, J., and Pollard, T. D., Purification of a cortical complex containing two unconventional actins from *Acanthamoeba* by affinity chromatography on profilin-agarose. J. Cell Biol. *127* (1994) 107–115.

85 McCaffrey, G., and Vale, D. V., Identification of a kinesin-like microtubule-based motor protein in *Dictyostelium discoideum*. EMBO J. *8* (1989) 3229–3234.

86 McRobbie, S. J., and Newell, P. C., Changes in actin associated with the cytoskeleton following chemotactic stimulation of *Dictyostelium discoideum*. Biochem. biophys. Res. Commun. *115* (1985) 351–359.

87 Murgia, I., Maciver, S. K., and Morandini, P., An actin-related protein from *Dictyostelium discoideum* is developementally regulated and associated with mitochondria. FEBS Lett. *360* (1995) 235–241.

88 Nachmias, V. T., Small actin-binding proteins: the $\beta$-thymosin family. Curr. Opin. Cell Biol. *5* (1993) 56–62.

89 Noegel, A. A., Rapp, S., Lottspeich, F., Schleicher, M., and Stewart, M., The *Dictyostelium* gelation factor shares a putative actin binding site with $\alpha$-actinins and dystrophin and also has a rod domain containing six 100-residue motifs that appear to have a cross-beta conformation. J. Cell Biol. *109* (1989) 607–618.

90 Noegel, A. A., Gerisch, G., Lottspeich, F., and Schleicher, M., A protein with homology to the C-terminal repeat sequence of *Octopus* rhodopsin and synaptophysin is a member of a multigene family in *Dictyostelium discoideum*. FEBS Lett. *226* (1990) 118–122.

91 Okazaki, K., and Yumura, S., Differential association of three actin-bundling proteins with microfilaments in amoebae. Eur. J. Cell Biol. *66* (1995) 75–81.

92 Ostrow, B. D., Chen, P., and Chisholm, R. L., Expression of a myosin regulatory light chain phosphorylation site mutant complements the cytokinesis and developmental defects of *Dictyostelium* RMCL null cells. J. Cell Biol. *127* (1994) 1945–1955.

93 Owen, C. H., DeRosier, D. J., and Condeelis, J., Actin crosslinking protein EF-1 $\alpha$ of *Dictyostelium discoideum* has a unique bonding rule that allows square-packed bundles. J. Struct. Biol. *109* (1992) 248–254.

94 Pantaloni, D., and Carlier, M. F., How profilin promotes actin filament assembly in the presence of thymosin $\beta$4. Cell *75* (1993) 1007–1014.

95 Pardo, J. V., Pittenger, M. F., and Craig. S. W., Subcellular sorting of isoactins: Selective association of G-actin with skeletal muscle mitochondria. Cell *32* (1983) 1093–1103.

96 Podolski, J. L., and Steck, T. L., Length distribution of F-actin in *Dictyostelium discoideum*. J. biol. Chem. *265* (1990) 1312–1318.

97 Pollard, T. D., Almo, S., Quirk, S., Vinson, V., and Lattman, E. E., Structure of actin binding proteins: insights about function at atomic resolution. A. Rev. Cell Biol. *10* (1994) 207–249.

98 Pollenz, R. S., Chen, T.-L. L., Trivinos-Lagos, L., and Chisholm, R. L., The *Dictyostelium* essential light chain is required for myosin function. Cell *69* (1992) 951–962.

99 Romans, P., and Firtel, R. A., Organization of the actin multigene family of *Dictyostelium discoideum* and analysis of variability in the protein coding regions. J. molec. Biol. *186* (1985) 321–335.

100 Romans, P., Firtel, R. A., and Saxe III, C. L., Gene-specific expression of the actin multigene family of *Dictyostelium discoideum*. J. molec. Biol. *186* (1985) 337-355.

101 Rubino, S., Unger, E., Fogu, G., and Cappuccinelli, P., Effect of microtubule inhibitors on the tubulin system of *Dictyostelium discoideum*. Zeitschrift für Allgemeine Mikrobiologie *22* (1982) 127–131.

102 Satterwhite, L. L., and Pollard, T. P., Cytokinesis, Curr. Opin. Cell Biol. *4* (1992) 43–52.

103 Sauterer, R. A., Eddy, R. J., Hall, A. L., and Condeelis, J., Purification and characterization of aginactin, a newly identified agonist-regulated actin capping protein from *Dictyostelium* amoebae. J. biol. Chem. *264* (1991) 24533–24539.

104 Scheel, J., Ziegelbauer, K., Kupke, T., Humbel, B. M., Noegel, A. A., Gerisch, G., and Schleicher, M., Hisactophilin, a histidine-rich actin-binding protein from *Dictyostelium discoideum*. J. biol. Chem. *264* (1989) 2832–2839.

105 Schleicher, M., Gerisch, G., and Isenberg, G., New actin-binding proteins from *Dictyostelium discoideum*. EMBO J. *3* (1984) 2095–2100.

106 Schleicher, M., and Noegel, A. A., Dynamics of the *Dictyostelium* cytoskeleton during chemotaxis. New Biol. *4* (1992) 461–471.

107 Schnuchel, A., Wiltscheck, R., Eichinger, L., Schleicher, M., and Holak, T. A., Structure of severin domain 2 in solution. J. molec. Biol. *247* (1995) 21–27.

108 Schroer, T. A., Fyrberg, E., Cooper, J. A., Waterston, R. H., Helfman, D., Pollard, T. D., and Meyer, D. I., Actin-related protein nomenclature and classification. J. Cell Biol. *127* (1994) 1777–1778.

109 Schweiger, A., Mihalache, O., Ecke, M., and Gerisch, G., Stage-specific tyrosine phosphorylation of actin in *Dictyostelium discoideum* cells. J. Cell Sci. *102* (1992) 601–609.
110 Shariff, A., and Luna, E. J., *Dictyostelium discoideum* plasma membranes contain an actin-nucleating activity that requires ponticulin, an integral membrane glycoprotein. J. Cell Biol. *110* (1990) 681–692.
111 Shiina, N., Gotoh, Y., Kubomura, N., Iwamatsu, A., Nishida, E., Microtubule severing by elongation factor 1α. Science *266* (1994) 282–235.
112 Spudich, J. A., In pursuit of myosin function. Cell Regul. *1* (1989) 1–11.
113 Stadler, J., Bauer, G., and Gerisch, G., Acylation in vivo of the contact site A glycoprotein and of other membrane proteins in *Dictyostelium discoideum*. FEBS Lett. *172* (1984) 326–330.
114 Stadler, J., Gerisch, G., Bauer, G., and Deppert, W., In vivo acylation of *Dictyostelium* actin with palmitic acid. EMBO J. *4* (1985) 1153–1156.
115 Stossel, T. P., The machinery of blood cell movements. Blood *84* (1994) 367–379.
116 Stratford, C. A., and Brown, S. S., Isolation of an actin-binding protein from membranes of *D. discoideum*. J. Cell Biol. *100* (1985) 727–735.
117 Theriot, J. A., and Mitchison, T. J., The three faces of profilin. Cell *75* (1993) 835–838.
118 Titus, M. A., Wessels, D., Spudich, J. A., and Soll, D., The unconventional myosin encoded by the *MYOA* gene plays a role in *Dictyostelium* motility. Molec. Biol. Cell *4* (1993) 233–246.
119 Titus, M. A., Kuspa, A., and Loomis, W., Discovery of myosin genes by physical mapping in *Dictyostelium*. Proc. natl Acad. Sci. USA *91* (1994) 9446–9450.
120 Trivinos-Lagos, L., Ohmachi, T., Albrigtson, C., Burns, R. G., Ennis, H. L., and Chisholm, R. L., The highly divergent α- and β-tubulins from *Dictyostelium discoideum* are encoded by sinlge genes. J. Cell Sci. *105* (1993) 903–911.
121 Van Haastert, P. J. M., Janssens, P. M. W., and Erneux, C., Sensory transduction in eukaryotes. A comparison between *Dictyostelium* and vertebrate cells. Eur. J. Biochem. *195* (1991) 289–303.
122 Vandekerckhove, J., and Weber, K., Vegetative *Dictyostelium* cells containing 17 actin genes express a single major actin. Nature, Lond. *284* (1980) 475–477.
123 Varnum, B., and Soll, D. R., Effects of cAMP on single cell motility in *Dictyostelium* . J. Cell Biol. *99* (1984) 1151–1155.
124 Weiner, O. H., Murphy, J., Griffiths, G., Schleicher, M., and Noegel, A. A., The actin-binding protein comitin (p24) is a component of the Golgi apparatus. J. Cell Biol. *123* (1993) 23–34.
125 Wessels, D., Murray, J., Jung, G., Hammer, J., and Soll, D., Myosin IB null mutants of *Dictyostelium* exhibit abnormalities in motility. Cell Motil. Cytoskel. *20* (1991) 301–315.
126 White, E., Tolbert, E. M, and Katz, E. R., Identification of tubulin in *Dictyostelium discoideum*: characterization of some unique properties. J. Cell Biol. *97* (1983) 1011–1019.
127 Witke, W., Schleicher, M., Lottspeiche, F., and Noegel A., Studies on the transcription, translation, and structure of α-actinin in *Dictyostelium discoideum*. J. Cell Biol. *103* (1986) 969–975.
128 Witke, W., Schleicher, M., and Noegel, A. A., Redundancy in the microfilament system: abnormal development of *Dictyostelium* cells lacking two F-actin crosslinking proteins. Cell *68* (1992) 53–62.
129 Wuestehube, L. J., and Luna, E. J., F-actin binds to the cytoplasmic surface of ponticulin, a 17-kD integral glycoprotein from *Dictyostelium discoideum*. J. Cell Biol. *105* (1987) 1741–1751.
130 Wuestehube, L. J., Chia, C. P., and Luna, E. J., Indirect immunofluorescence localization of ponticulin in motile cells. Cell Motil. Cytoskel. *13* (1989) 245–263.
131 Yamamoto, K., Pardee, J. D., Reidler, J., Stryer, L., and Spudich, J. A., Mechanism of interaction of *Dictyostelium* severin with actin filaments. J. Cell Biol. *95* (1982) 711–719.
132 Yang, F., Demma, M., Warren, V., Dharmawardhane, S., and Condeelis, J., Identification of actin-binding protein from *Dictyostelium* as elongation factor 1α. Nature, Lond. *347* (1990) 494–496.
133 Yin, H. L., Janmey, P. A., and Schleicher, M., Severin is a gelsolin prototype. FEBS Lett. *264* (1990) 78–80.
134 Zhu, Q., and Clarke, M., Association of calmodulin and an unconventional myosin with the contractile vacuole complex of *Dictyostelium discoideum*. J. Cell Biol. *118* (1992) 347–358.
135 Zigmond, S. H., Furukawa, R., and Fechheimer, M., Inhibition actin filament depolymerization by the *Dictyostelium* 30,000-D actin-bundling protein. J. Cell Biol. *119* (1992) 559–567.