# Ponticulin Is the Major High Affinity Link between the Plasma Membrane and the Cortical Actin Network in *Dictyostelium*

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*Abstract.* Interactions between the plasma membrane and underlying actin-based cortex have been implicated in membrane organization and stability, the control of cell shape, and various motile processes. To ascertain the function of high affinity actin-membrane associations, we have disrupted by homologous recombination the gene encoding ponticulin, the major high affinity actin-membrane link in *Dictyostelium discoideum* amoebae. Cells lacking detectable amounts of ponticulin message and protein also are deficient in high affinity actin-membrane binding by several criteria. First, only 10–13% as much endogenous actin cosediments through sucrose and crude plasma membranes from ponticulin-minus cells, as compared with membranes from the parental strain. Second, purified plasma membranes exhibit little or no binding or nucleation of exogenous actin in vitro. Finally, only 10–30% as much endogenous actin partitions with plasma membranes from ponticulin-minus cells after these cells are mechanically unroofed with polylysine-coated coverslips.

The loss of the cell's major actin-binding membrane protein appears to be surprisingly benign under laboratory conditions. Ponticulin-minus cells grow normally in axenic culture and pinocytose FITC-dextran at the same rate as do parental cells. The rate of phagocytosis of particles by ponticulin-minus cells in growth media also is unaffected. By contrast, after initiation of development, cells lacking ponticulin aggregate faster than the parental cells. Subsequent morphogenesis proceeds asynchronously, but viable spores can form. These results indicate that ponticulin is not required for cellular translocation, but apparently plays a role in cell patterning during development.

DIRECT or indirect high affinity associations between actin and the plasma membrane in the so-called "membrane skeleton" are thought to control the shapes of erythrocytes and platelets and to stabilize sites of cell–cell and cell–substrate attachment in body tissues (for recent reviews see reference 31). Actin assembly at or near the plasma membrane also has been implicated in directed movements of single cells and in pattern formation during development (for recent reviews see references 10, 12, 14, 36, 54, 60, 70). However, little definitive information is available about the role of the membrane skeleton in such dynamic cell processes. For instance, alternative mechanisms, mediated primarily by changes in the cortical cytoskeleton, including force production by myosin motors, osmotic forces, and biased Brownian motion, have been proposed to drive cell translocation (10, 22, 48, 50, 58). Thus, the role of the membrane skeleton in pseudopod extension, membrane stabilization, and adhesion of motile cells is unclear.

Amoebae of the cellular slime mold, *Dictyostelium discoideum,* constitute an excellent system for the elucidation of membrane skeleton function. These cells ingest both particles and fluid-phase nutrients during log-phase growth, form multicellular aggregates during their well-characterized developmental cycle, and are highly motile during both growth and development (for review see references 39, 40, 59). The behavior, morphology, and biochemistry of *Dictyostelium* engaged in motile processes are strikingly like those of the best-characterized mammalian amoeboid cells, such as polymorphonuclear leukocytes (6, 11, 63). Detailed information also is available about the composition of both the *Dictyostelium* plasma membrane and cortical cytoskeleton (for reviews see references 27, 39, 41, 55), and actin-membrane interactions have been studied extensively (44). Finally, this haploid organism is readily amenable to gene disruption by homologous recombination, an approach that has proved useful for the determination of cytoskeletal protein functioning in *Dictyostelium* and other genetic systems (for reviews see references 5, 18, 21, 25, 55, 65).

Both high affinity binding of actin to the *Dictyostelium* plasma membrane and membrane-mediated actin nucleation appear to be primarily attributable to ponticulin (8, 9, 43), a 17-kD transmembrane glycoprotein with a unique structure (31). Because ponticulin is anchored in the plasma membrane, it is expected to play a major role in the structure of

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the membrane skeleton but to have essentially no direct effect on the composition of the cortical cytoskeleton. However, ponticulin is almost certainly not the only component of the *Dictyostelium* membrane skeleton. Other actin-binding proteins, including histactophilin (53), coronin (15), myosins I (23, 35), and a spectrin-like protein (3), also have been localized at or near the plasma membrane in these cells and may function at the cytoskeleton-membrane interface.

As the first step towards the determination of the role of ponticulin relative to that of other potential membrane skeletal proteins, we have generated loss-of-function mutants in which the single copy ponticulin gene has been disrupted by homologous recombination. *Dictyostelium* amoebae with a disrupted ponticulin gene lack detectable ponticulin message and protein. Plasma membranes from these cells also lack essentially all actin nucleation activity and stable high affinity binding for actin in vitro. In addition, electron microscopy of mechanically unroofed cells demonstrates that ponticulin is responsible for most of the binding between cortical actin and the plasma membrane in vivo.

The loss of this major high affinity actin-membrane interaction is surprisingly benign under laboratory conditions. Cells lacking ponticulin grow pinocytose FITC-labeled dextran, and phagocytose particles at rates not significantly different from rates observed for the parental AX3K cells. However, cells lacking ponticulin do demonstrate developmental abnormalities. Although ponticulin-minus cells aggregate into mounds even faster than do the parental cells, ponticulin-minus cells can become stalled at this stage for prolonged and variable times. These mounds do eventually complete development, culminating in fruiting bodies with viable spores.

Our results indicate that ponticulin is a major component of the *D. discoideum* membrane skeleton, the first amoeboid membrane skeleton to be so characterized. This protein is apparently responsible for most of the high affinity binding between actin and the plasma membrane in vivo, as well as in vitro. Nevertheless, our results show that the high affinity actin-binding and nucleating activities mediated by ponticulin are dispensable during directed cell movement. On the other hand, ponticulin function is required for normal *Dictyostelium* aggregation and development, implying an important role for the membrane skeleton during pattern formation and morphogenesis.

## Materials and Methods

### Ponticulin-minus Mutants

*D. discoideum* amoebae, strain AX3K, were grown as described in the accompanying paper (31). To make the gene disruption construct, the neomycin resistance cassette was excised with EcoRV from pDneoII (67), a generous gift of Dr. Angelika Noegel, and ligated into the AflII site of pGB (nt 9 of the coding region of *ponA*; see Fig. 1), which had been filled in with Klenow. Plasmid DNA was isolated from Sure® cells, and the orientation of the cassette with respect to the *ponA* gene was determined by restriction analysis. In pPonR, the *neo* promoter is oriented in the same direction as the ponticulin promoter. The alternate construct, pPonL, has the *neo* promoter oriented in the opposite direction. Digestion of these plasmids with BglII and XhoI generated a linear DNA fragment containing the disrupted ponticulin gene and ~2.0 kb of upstream sequence. *Dictyostelium* ($10^6$ cells) were transformed with 5 μg of either pPonR or pPonL at 25 μF, 1,250 V in a 0.4-cm cuvette with a Bio-Rad (Hercules, CA) Gene Pulser (38). After recovery overnight in HL-5 media (10 ml), ~$5 \times 10^5$ cells per plate were grown for 20 d in media containing 10 μg/ml G418. DNA isolated from transformed colonies was tested for the existence of the undisrupted *ponA* gene by PCR with oligonucleotides corresponding to flanking genomic sequences: nt 311-332 in pGB (~200 bp upstream of the 5'-BglIII site) and nt 398-420 of *ponA* (10–32 bp downstream of the 3'-HindIII site). Clone Tf1.1 was generated using pPonR, and clone Tf24.1 was generated using pPonL. Both cell lines were subcloned twice by limiting dilution in HL-5 media. Suspension cultures of ponticulin-minus and control AX3K cells were started from frozen stocks of the original subclones every 2–5 wk.

### Southern and Northern Blots

Nucleic acids were transferred to Duralon (Stratagene, La Jolla, CA) and probed with a riboprobe to pPCR as described in the accompanying manuscript (31).

### Membrane Preparations, Immunoblots, and Actin Blot Overlays

Purified plasma membranes were prepared from log-phase cells by the Con A stabilization/Triton extraction method (Con A/Triton plasma membranes) (42). Ponticulin was visualized either with $^{125}$I-labeled F-actin (8) or with R67 IgG and $^{125}$I-protein A (New England Nuclear Dupont, Wilmington, DE), as described in the accompanying paper (31).

### Actin-binding and Nucleation Assays

Binding assays were carried out as described by Schwartz and Luna (56). Each sample consisted of 50 μl of polymerization buffer containing 50 μg/ml of Con A/Triton plasma membranes, 1 mg/ml ovalbumin, and varying amounts of actin:gelsolin in a 50:1 molar ratio. After a 1-h incubation at room temperature, samples were centrifuged through 300 μl of 10% (wt/vol) sucrose in polymerization buffer. The tubes were frozen and cut, and the supernatants and pellets were counted in a gamma counter.

Actin nucleation assays were performed as described by Shariff and Luna (57). Each assay contained 20 μg of Con A/Triton plasma membranes and 3 μM pyrene actin in a final volume of 0.5 ml. Fluorescence was measured using an excitation wavelength of 365 nm and an emission wavelength of 407 nm. The pyrene actin fluorescence was normalized from 0–10 and plotted vs time with 10 representing the fluorescence after 24 h of polymerization.

### Electron Microscopy

*Dictyostelium* amoebae, grown to 1–2 × $10^6$ cells/ml, were attached to coverslips and mechanically unroofed with a second, top coverslip as previously described for macrophages (29), except that cells were incubated at room temperature and cleaved in the presence of 15 mM Pipes, 6.25 mM Hepes, 10 mM EGTA, and 0.5 mM $MgCl_2$, 5 μM phallacidin, 42 nM leupeptin, 10 mM benzamidine, 0.123 mM aprotinin, pH 6.9. Top coverslips containing bound cell surfaces were fixed for 15 min in the same buffer containing 2% glutaraldehyde, extensively washed with distilled water, rapidly frozen on a liquid helium-cooled block, freeze-dried at −90°C, and rotary coated with 1.4 nm of tantalum-tungsten at 45° and with 2.5 nm of carbon at 90° without rotation in a Cressington (Watford, UK) CFE-50 Freeze Fracture Apparatus. Coverslip-adherent cell fragments also were incubated with 5 μM skeletal muscle myosin subfragment 1 for 10 min at room temperature, washed, fixed with 0.2% tannic acid, 1% glutaraldehyde, 10 mM sodium phosphate, pH 7.5, and frozen as described above. Replicas were processed and photographed at 100 kV in a JEOL (Peabody, MA) EX-1200 electron microscope, as described (29).

### Pinocytosis and Phagocytosis Assays

Rates of phagocytosis and pinocytosis were determined from the uptake of fluorescent beads (Fluoresbright Carboxy YG 1.0 μm beads, #15702, Polysciences, Warrington, PA) and FITC-labeled dextran (FD-70S; Sigma Chem. Co., St. Louis, MO), respectively, using the methods of Vogel (64), as modified by Chia and Luna (7). Briefly, ~$2 \times 10^6$/ml *Dictyostelium* were incubated for the designated times with ~$4 \times 10^8$ fluoresbrite beads per ml of filtered (0.45 μm) HL-5 growth media or with 2 mg/ml of FITC-dextran in filtered HL-5 media. Phagocytosis and pinocytosis were halted by rapid dilution into ice cold buffer, and external label was removed by centrifugation through 20% polyethylene glycol and washing with 50 mM sodium phosphate, pH 9.2. Label was liberated from the cells with 0.2% Triton X-100 (25°C, 1 h) and measured at 25°C on a Perkin-Elmer

(Norwalk, CT) LS-3 spectrofluorimeter. Amounts of internalized dextran and beads were determined by comparison with standard curves.

### *Developmental Time Course*

The *Dictyostelium* developmental cycle was initiated and followed as described in the accompanying manuscript (31). Photographs were taken with Kodak T-Max 400 film using an Olympus (New Hyde Park, NY) SZH10 Research Stereo Microscope equipped with a polarizing filter.

## *Results*

### *Ponticulin-minus Cells*

Cells with a disrupted ponticulin gene were generated by homologous recombination using a construct containing a neomycin resistance cassette (Fig. 1). After selection with G418, transformants lacking a 2.5-kb PCR product diagnostic of the single-copy *ponA* gene were subcloned by limiting dilution. Two of these independent transformants (Tfl.1 and Tf24.1) were characterized further. Analysis of genomic DNA demonstrated that both mutants lack the ~4-kb BglII fragment containing the endogenous *ponA* gene (Fig. 2 *A*). Instead, both ponticulin-minus cell lines contain BglII fragment of ⩾ 6 kb, indicating that the pPon-neo constructs integrated into the region of the *ponA* gene. As is typical of constructs containing the *neo* cassette (see references 13, 16, 17 for other examples), multiple integration events occurred in each transformed cell line. TFl.1 and Tf24.1 cells have ~11 and ~10 copies, respectively, of the disruption construct, as determined by scanning densitometry with a Phosphorimager SF (Molecular Dynamics, Sunnyvale, CA). Tfl.1 and Tf24.1 cells also lack detectable amounts of the ~0.8-kb ponticulin message (Fig. 2 *B*).

As expected, Tfl.1 and Tf24.1 cell lines contain no ponticulin detectable by either antigenic cross-reactivity or binding to F-actin (Fig. 3). No signal at the position of ponticulin is observed on immunoblots probed with R67 IgG (Fig. 3, *left*) or at the membrane after immunofluorescence microscopy with this antibody (not shown). Plasma membranes from Tfl.1 and Tf24.1 cells also lack the major 17-kD actin-binding protein observed on blot overlays with $^{125}$I-labeled F-actin (Fig. 3, *right*), further confirming that the ponticulin gene has been disrupted (8). These ponticulin-minus cell lines also lack the minor 15 and 19-kD actin-binding membrane proteins recognized by R67 IgG and by $^{125}$I-labeled F-actin on blot overlays, supporting the previous suggestion

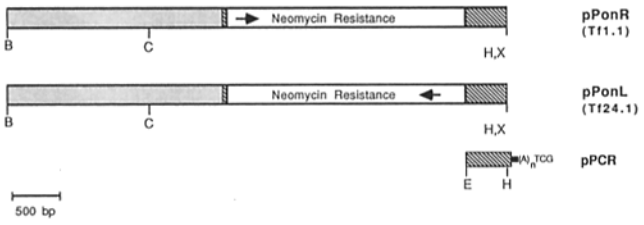


*Figure 1.* Gene disruption constructs. (*Diagonal pattern*) The coding sequence for ponticulin (*ponA*); (*stippling*) flanking sequence; and (*white*) neomycin resistance cassette. Arrows denote the direction of the orientation of the neomycin resistance cassette in ponticulin-minus mutants, Tfl.1 and Tf24.1. The construct pPCR containing the coding region of the *ponA* gene was used to analyze Southern and Northern blots of the ponticulin-minus cells. *B*, BglII; *C*, ClaI; *E*, EcoRI; *H*, HindIII; *X*, XhoI.

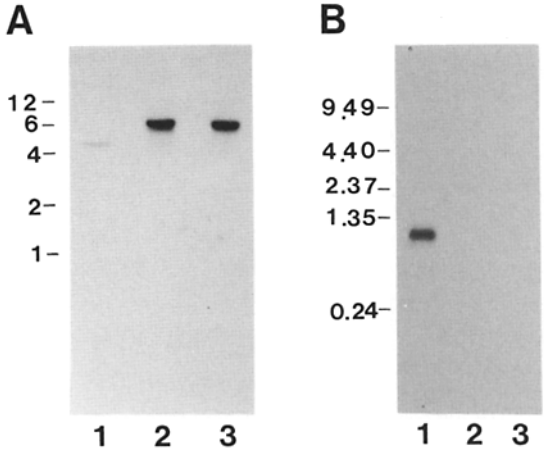


*Figure 2.* Mutant cells contain neither an intact *ponA* gene nor detectable amounts of the ponticulin message. (*A*) Southern blot. Genomic *Dictyostelium* DNA (10 μg/lane) from AX3K cells (lane *1*), Tfl.1 cells (lane *2*), and Tf24.1 cells (lane *3*) was digested with BglII and probed with a riboprobe corresponding to pPCR. The blot was exposed for 24 h with one intensifying screen at −80°C. (*B*) Northern blot. Total RNA (10 μg/lane) isolated from AX3K cells (lane *1*), Tfl.1 cells (lane *2*), and Tf24.1 cells (lane *3*) was probed with a riboprobe corresponding to pPCR and exposed to film for 20 h with one intensifying screen at −80°C. Standards in kb, as shown.

that these polypeptides are differentially glycosylated isoforms of ponticulin (8).

### *Characterization of Actin-Membrane Associations in Ponticulin-minus Cells*

Because ponticulin appears to be the major actin-binding

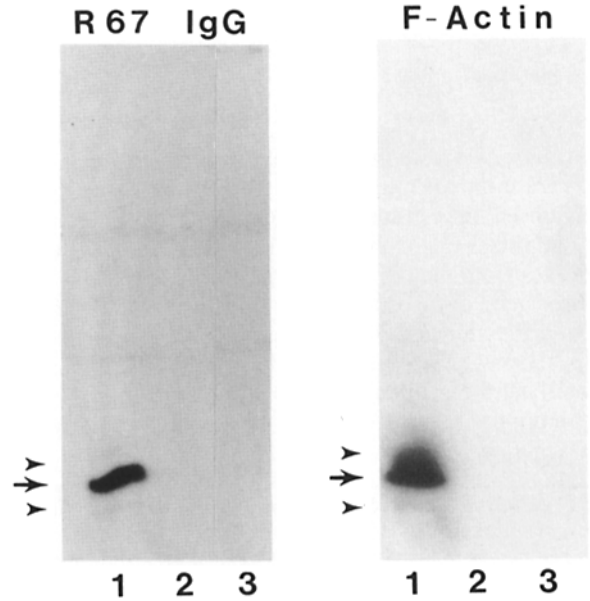


*Figure 3.* Mutant cells lack detectable ponticulin. (*Left*) Immunoblot of whole cells. (*Right*) $^{125}$I-labeled F-actin blot overlay of isolated plasma membranes. AX3K (lanes *1*), Tfl.1 (lanes *2*), and Tf24.1 (lanes *3*). Gels were loaded with ~5 × $10^6$ cells/lane (*left*) or with 30 μg Con A/Triton plasma membranes/lane (*right*). Arrows indicate the position of ponticulin at 17-kD; arrowheads indicate the positions of minor 15-kD and 19-kD ponticulin isoforms.

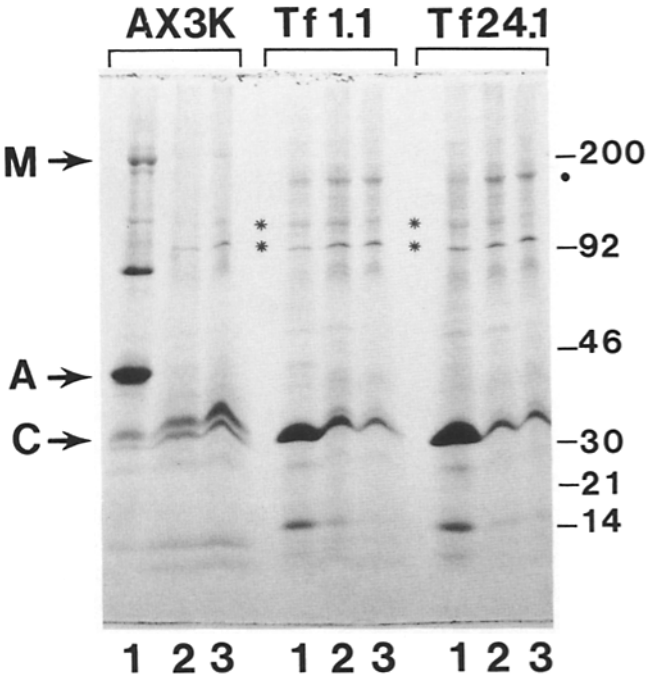


*Figure 4.* Endogenous actin readily dissociates from membranes without ponticulin. Coomassie blue-stained gel of samples (50 μg/lane) taken during the plasma membrane preparation. (Lanes *1*) Samples taken after the cells have been stabilized with Con A, lysed with Triton X-100, and stabilized membranes centrifuged through a sucrose gradient. In the parental cells (*AX3K*), there are three major protein bands: myosin (*M*), actin (*A*), and Con A (*C*) (42). (lanes *2*) Membranes after treatment with alpha-methylmannoside to remove most of the extracellular Con A. (lanes *3*) Highly purified plasma membranes after low ionic strength dialysis and centrifugation on a second sucrose gradient. Integral proteins of ~100- and ~130-kD characteristic of *Dictyostelium* plasma membranes (*) and an unknown ~185-kD protein variably enriched in membranes from ponticulin-minus cells (•) are evident in the purified plasma membrane fractions.

protein in the *Dictyostelium* plasma membrane, we examined the ponticulin-minus mutants for defects in actin-membrane interactions. Unlike crude plasma membranes isolated from the parental cell line (Fig. 4, *AX3K*, lane *1*), crude plasma membranes from the ponticulin-minus cells contained little associated actin or myosin (Fig. 4, *Tf1.1* and *Tf24.1*). Quantification of the amount of Coomassie blue-staining material at ~43 kD on SDS-polyacrylamide gels indicated that the amount of actin cosedimenting with these crude membranes averaged no more than 10–13% of the amount normally observed (Table I). Due to the presence of membrane polypeptides other than actin at this molecular mass (27, 42), these numbers are upper estimates of the amounts of cosedimenting actin. Crude membranes from cells lacking ponticulin did contain a number of major polypeptides, including prominent bands at ~100 kD and ~130 kD (*asterisks*), that apparently correspond to known integral plasma membrane glycoproteins (27, 42). After the removal of most of the Con A used to stabilize the plasma membrane (Fig. 4, lanes *2*), removal of residual actin by low ionic strength dialysis, and centrifugation into a second sucrose gradient, the 100- and 130-kD polypeptides were evident in all three preparations of highly purified plasma membranes (Fig. 4, lanes *3*).

Besides these similarities, differences in the membrane protein profiles from ponticulin-minus cells were sometimes observed (Fig. 4). For instance, plasma membranes from ponticulin-minus cells were variably enriched in a ~185-kD polypeptide that was either absent from or much less evident in plasma membranes from AX3K cells (Fig. 4, *closed circle*). Microsequence analyses of tryptic peptides from this polypeptide show no significant similarities to any protein in protein sequence data banks (Leszyk, J., A. L. Hitt, and E. J. Luna, unpublished data). These observations raise the possibility that the absence of ponticulin may induce the upregulation of the 185-kD protein or may stimulate its redistribution from another cellular compartment.

Consistent with the low amounts of copurifying endogenous actin, highly purified plasma membranes from ponticulin-minus cells did not bind actin in sedimentation assays (Fig. 5, Tf1.1 data not shown). These membranes also did not efficiently nucleate polymerization of 3 μM pyrene-labeled actin, as judged by a comparison of the maximal rates of polymerization (Fig. 6). However, the lag time before onset of polymerization in the presence of ponticulin-minus membranes was sometimes observed to be shorter than that observed for actin alone, consistent with a small and variable

*Table I. Relative Amounts of Actin Cosedimenting with Plasma Membrane*

| Preparation | AX3K | Tf1.1 | Tf24.1 |
|---|---|---|---|
| 1 | 100% | 8.2% | 7.6% |
| 2 | 100% | 17.1% | 16.1% |
| 3 | 100% | 13.7% | 6.7% |
| | Average ± SD: | 13.0 ± 4.5% | 10.1 ± 5.2% |

Estimates of the relative amounts of actin associated with each membrane preparation were determined from scans of the 43-kD region of Coomassie blue-stained SDS-polyacrylamide gels.

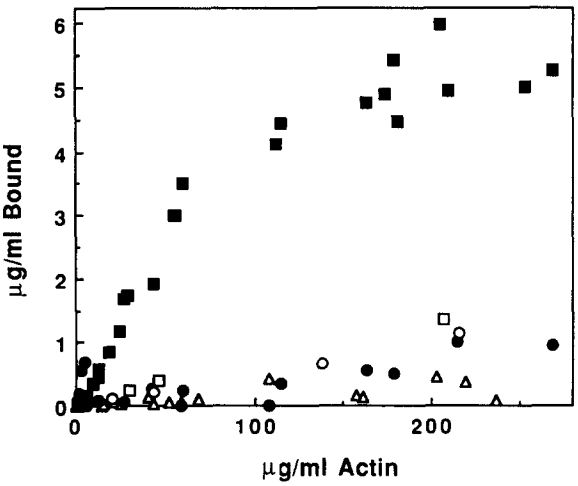


*Figure 5.* Ponticulin is required for high affinity actin binding to isolated plasma membranes in sedimentation assays. Cosedimentation of polymerizing actin with highly purified plasma membranes (50 μg/ml). Parental (AX3K) membranes (■), Tf24.1 membranes (●), heat-treated AX3K membranes (□), heat-treated Tf24.1 membranes (○), and no membranes (△).

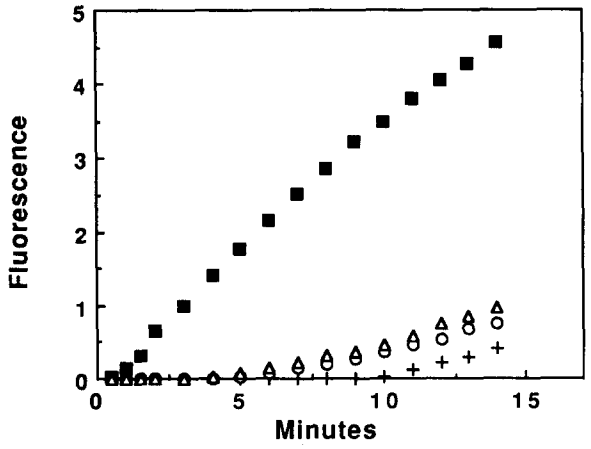


*Figure 6.* Ponticulin is required for the actin nucleation activity of isolated *Dictyostelium* plasma membranes. Rate of polymerization of pyrene actin (3 μM total, 10% pyrene-labeled) in the presence of highly purified plasma membranes (40 μg/ml). Parental (AX3K) membranes (■), Tfl.1 membranes (△), Tf24.1 membranes (○), and no membranes (+).

amount of non-ponticulin-mediated nucleation. In both the actin-binding and nucleation assays, the specific activities of the membranes from the ponticulin-minus cell lines were essentially the same as that of heat-denatured membranes from AX3K cells (56, 57). Thus, ponticulin appears to be the major protein responsible for membrane-mediated actin nucleation and for stable, high affinity binding of actin filaments to the *Dictyostelium* plasma membrane.

Electron microscopy of cortices from mechanically unroofed cells supported and extended the conclusion that ponticulin is required for attachment of the actin-based cortex to the plasma membrane (Figs. 7 and 8). In marked contrast to the cytoplasmic surfaces of the parental AX3K cells, which were associated with a dense three-dimensional mat of actin filaments (Fig. 7), much less actin remained bound to membranes from cells lacking ponticulin (Fig. 8). Treatment with myosin subfragment 1 confirmed that the beaded, relatively straight filaments and filament bundles in these micrographs are actin (not shown). In rough agreement with a previous estimate of ~0.2 μm (51), these membrane-associated actin filaments ranged from ~0.16 to ~0.60 μm in length (Table II), although filament length distributions varied considerably among the different cell lines. This variability appeared to be independent of the presence or absence of ponticulin because membrane-associated actin filaments in cells lacking ponticulin could be either longer (Tfl.1) or shorter (Tf24.1) on average than those observed in control AX3K cells.

Membranes from unroofed ponticulin-minus cells exhibited less than a third as many filaments per square micrometer as were observed on the surfaces of membranes from the parental cells (Table II). Although the filament density on AX3K cell membranes reported in Table II is probably an underestimate, a decrease of 65–90% in the total amount of actin tightly bound to the plasma membrane also is consistent with the observed reduction of membrane-associated actin in similarly prepared samples stained with rhodamine-phalloidin (Strassel, C., A. L. Hitt, and E. J. Luna, unpublished observations). Despite the dramatic differences in the amount of membrane-associated actin, no consistent morphological differences between parental and ponticulin-minus cells were apparent in low and high power electron micrographs (Aghajanian, J., A. L. Hitt, and E. J. Luna, unpublished data).

Interestingly, the decrease in the amount of actin bound to Tfl.1 and Tf24.1 plasma membranes revealed additional structural features that were presumably present, but obscured, at the cytoplasmic surfaces of membranes from cells containing ponticulin. For instance, the sparse actin filaments on membranes lacking ponticulin often appeared to be laterally associated with flexible strands with diameters of ~3 nm (Fig. 8, *arrows*) and to terminate in globular structures (Fig. 8, *open arrows*). Other features of the cytoplasmic membrane surface, such as coated pits (Fig. 8, *arrowheads*), uncoated membrane invaginations (Fig. 8, *asterisk*), and novel ladderlike arrays (~17 nm wide and ~40 nm long) composed of parallel rows of ~8-nm particles (Fig. 8, *circles*), also became conspicuous in the absence of the dense actin cover.

### *Physiological Characterization of Cells Lacking Ponticulin*

Because actin-membrane interactions are believed to play an important role in many cellular processes, including endocytosis, cellular translocation and adhesion, we screened the mutant cell lines for gross defects in structure and biological activities. Initial observations of the ponticulin-minus cell lines indicated that these cells grew at the same rate as the parental AX3K cells, both in axenic medium (Fig. 9) and on bacterial lawns (not shown) under standard laboratory conditions at 21°C. Even at reduced temperatures, cells lacking ponticulin doubled at the same rate as the parental line (Fig. 9).

Depending upon conditions, *Dictyostelium* amoebae obtain sustenance by internalizing external fluid (pinocytosis) or solid particles (phagocytosis). In nutrient media, AX3K cells primarily ingest fluid through clathrin-coated pits (46, 47), whereas phagocytosis, a process that is dependent on the actin-based cytoskeleton (24, 34), predominates in the presence of bacteria or particulate matter. As expected from the axenic growth curves (Fig. 9), cells lacking ponticulin exhibited pinocytotic rates that were essentially the same as those observed for the parental cell line (Fig. 10 *A*). In growth media, phagocytosis of fluorescent beads (Fig. 10 *B*) and bacteria (data not shown) also did not differ significantly from the rate observed for the parental AX3K cells. These data indicate that, under these conditions, ponticulin is not required for phagocytosis or pinocytosis by *Dictyostelium* amoeba.

### *Development of Ponticulin-minus Cells*

Shortly after deprivation of nutrients, wild-type *Dictyostelium* amoebae initiate a well-characterized program of development in which the cells migrate together into aggregation centers, becoming visible mounds of as many as $10^5$ cells by 9–12 h after starvation (39, 40). These cells then coordinate to form a fruiting body consisting of a spore case elevated by a stalk 1–2-mm long. This developmental program is tightly regulated and involves a complicated series of molecular processes, including detection of nutrient

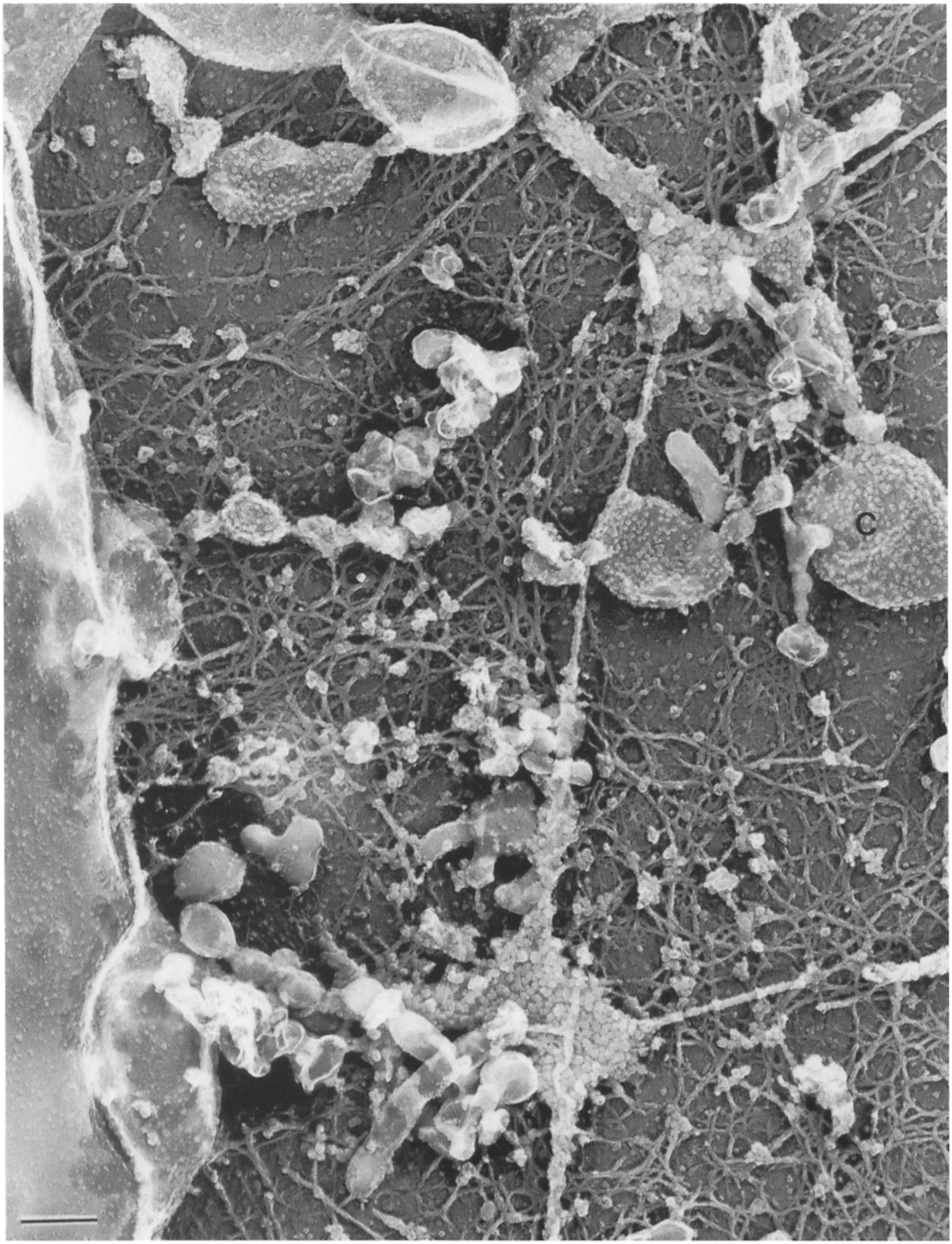


*Figure 7.* Electron micrograph showing the dense cover of actin filaments associated with the cytoplasmic surface of an unroofed AX3K cell. *C*, one component of the interconnected tubules and cisternae which constitute the contractile vacuole system in *Dictyostelium* (30). Bar, 0.2 $\mu$m.

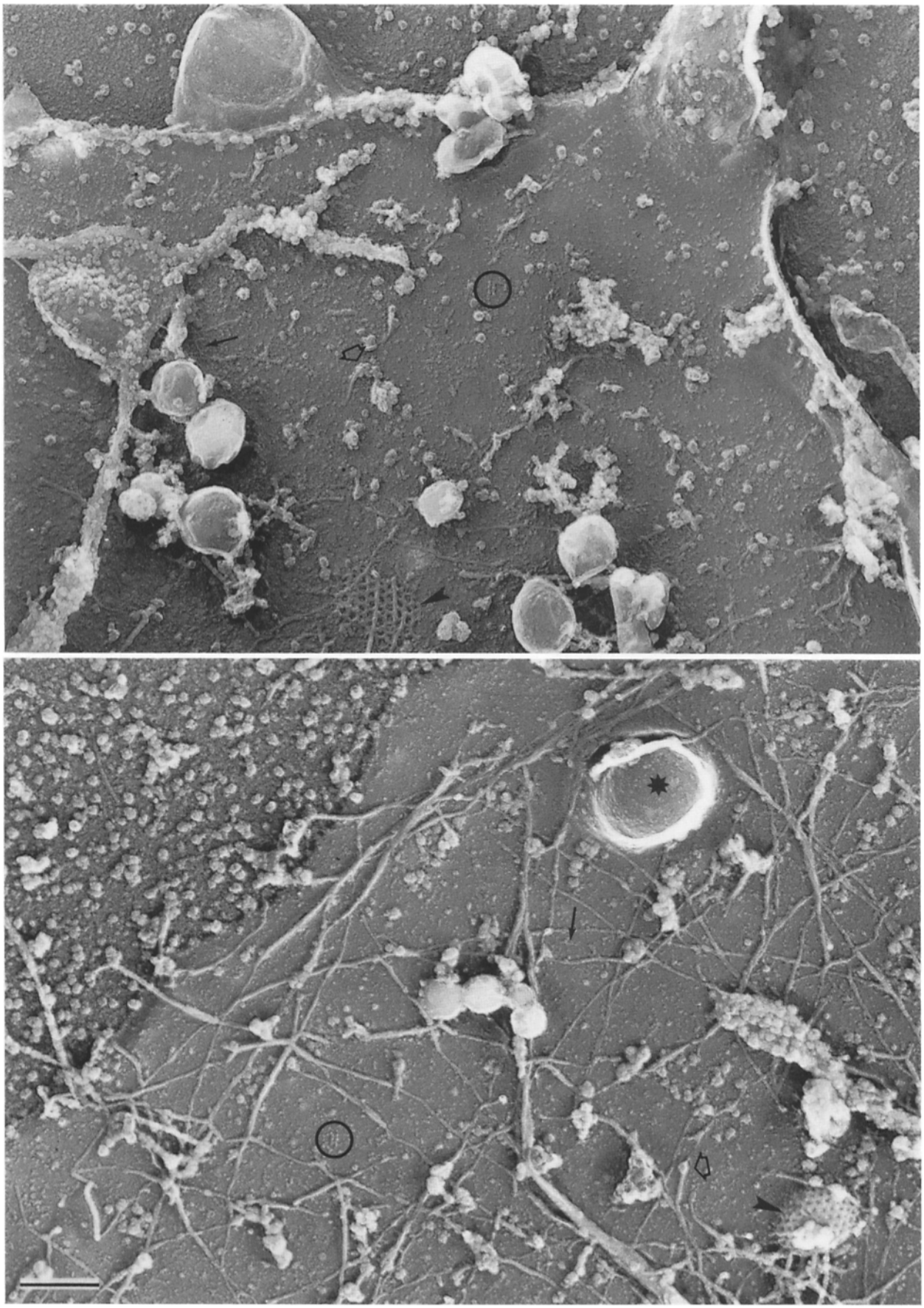

*Figure 8.* Electron micrographs showing the cytoplasmic surfaces of membrane fragments from ponticulin-minus cells. Both Tf24.1 cells (*upper panel*) and Tf.1.1 membranes (*lower panel*) contain no more than one-third the number of tightly associated actin filaments found on AX3K cell membranes (Fig. 7, Table II). Other features visible on these membrane surfaces include 3-nm actin-associated filaments (*arrows*), globular structures (*open arrows*), coated pits (*arrowheads*), uncoated membrane invaginations (*asterisk*), and previously undescribed 17 by 40 nm "ladders" comprised of 8-nm particles (*circles*).

*Table II. Actin Filaments on Plasma Membranes from Parental (AX3K) and Ponticulin-minus (Tf1.1, Tf24.1) Cells*

| Cell type | Filament density* | Average length | Total actin† |
|---|---|---|---|
| | ($n/\mu m^2$) | ($nm$) | ($\mu m/\mu m^2$) |
| AX3K | 106 ± 23 | 480 ± 26 | 50.9 ± 28 |
| Tf1.1 | 31 ± 9 | 596 ± 43 | 18.2 ± 1.3 |
| Tf24.1 | 33 ± 5 | 158 ± 13 | 5.2 ± 0.4 |

* Average filament density ± SEM from five randomly chosen areas of 1 $\mu m^2$ on the cytoplasmic surfaces of plasma membranes from different cells. In filament bundles, the total numbers of filaments ($n$) were estimated from the bundle diameters, assuming a two-, rather than a three-, dimensional array. This assumption probably results in an underestimate of the actin filament density, especially that observed on membranes from AX3K cells.
† Determined by multiplying the filament density and average filament length.

depletion, intercellular signaling, chemotaxis, cell–cell adhesion, and differentiation.

Although ponticulin-minus cells could stream, aggregate, and form stalks with viable spores, the developmental time course was aberrant. The loss of ponticulin appeared to accelerate formation of mounds but then to slow down the rest of the developmental process. Ponticulin-minus cells began to form mounds ~6 h after initiation of development (Fig. 11, *7.5 h*), or ~3 h before the onset of mound formation in parallel experiments with AX3K cells. Ponticulin-minus cells did continue along the normal developmental pathway but were temporally asynchronous. Although some of the ponticulin-minus mounds proceeded with a normal developmental time course to culminate in stalks and fruiting bodies at 24–28 h, immediately adjacent mounds often took much longer to develop (Fig. 11, *26 h*). For example, even at 46 h after initiation of development, many mounds (Fig. 11, *Tf1.1, arrowhead*) and pseudoplasmodia (Fig. 11, *Tf24.1, arrow*) of ponticulin-minus cells were still apparent. Thus, although ponticulin is not required for motility and chemotaxis of developing cells, it appears to play an important role in the temporal regulation of both early and late stages of development.

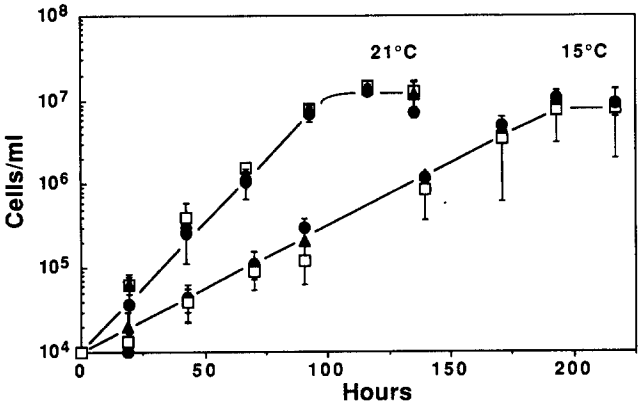


*Figure 9.* Ponticulin-minus cells grow normally in liquid culture. Growth of parental (AX3K) cells (□), Tf1.1 cells (▲), and Tf24.1 cells (●) at normal (21°C) and reduced (15°C) temperatures. Average cell densities ± SD are shown for triplicate points from a single experiment. Three data sets were obtained at each temperature with comparable results. Average doubling times at 21°C were 10.6 ± 0.5 h (AX3K), 10.6 ± 0.3. (Tf1.1), and 10.7 ± 0.9 h (Tf24.1); average doubling times at 15°C were 23.1 ± 2.3 h (AX3K), 22.6 ± 3.0 h (Tf.1.1), and 23.0 ± 2.5 h (Tf24.1).

## *Discussion*

In this paper, we report the generation and initial characterization of *Dictyostelium* amoebae in which the single copy gene encoding ponticulin is disrupted by homologous recombination (Figs. 1 and 2 *A*). These mutant cells contain neither detectable ponticulin message (Fig. 2 *B*) nor protein (Fig. 3). As expected from previous biochemical analyses (8, 57, 69), plasma membranes from ponticulin-minus cells lack essentially all high affinity-binding sites for endogenous (Fig. 4; Table I) and exogenous F-actin (Figs. 3, *right* and 5), as well as essentially all actin nucleation activity (Fig. 6). In addition, cytoplasmic surfaces of membranes from

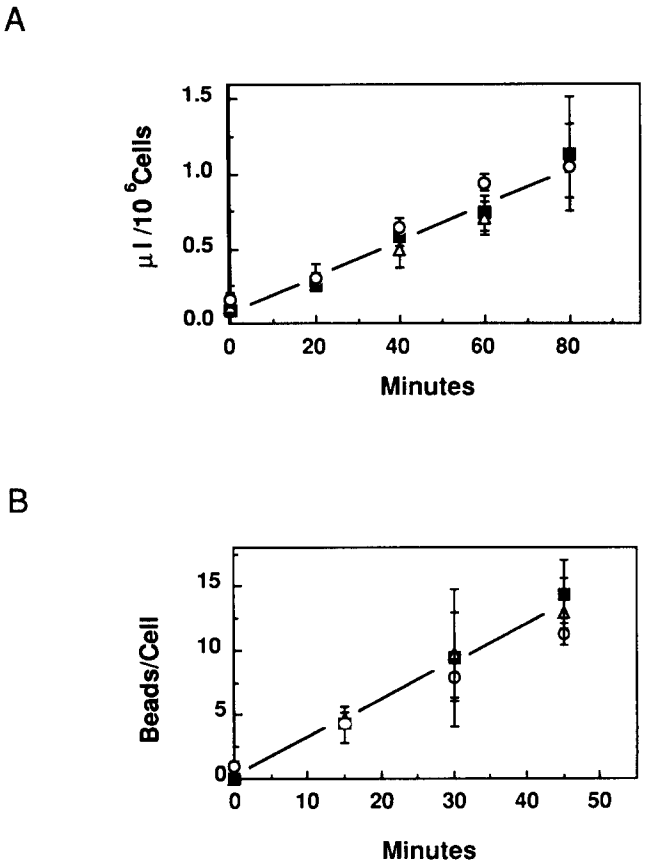


*Figure 10.* Endocytosis is normal in ponticulin-minus cells. (*A*) Pinocytosis of FITC-labeled dextran. (*B*) Phagocytosis of fluorescent beads. Parental (AX3K) cells (■), Tf1.1 cells (△), and Tf24.1 cells (○).

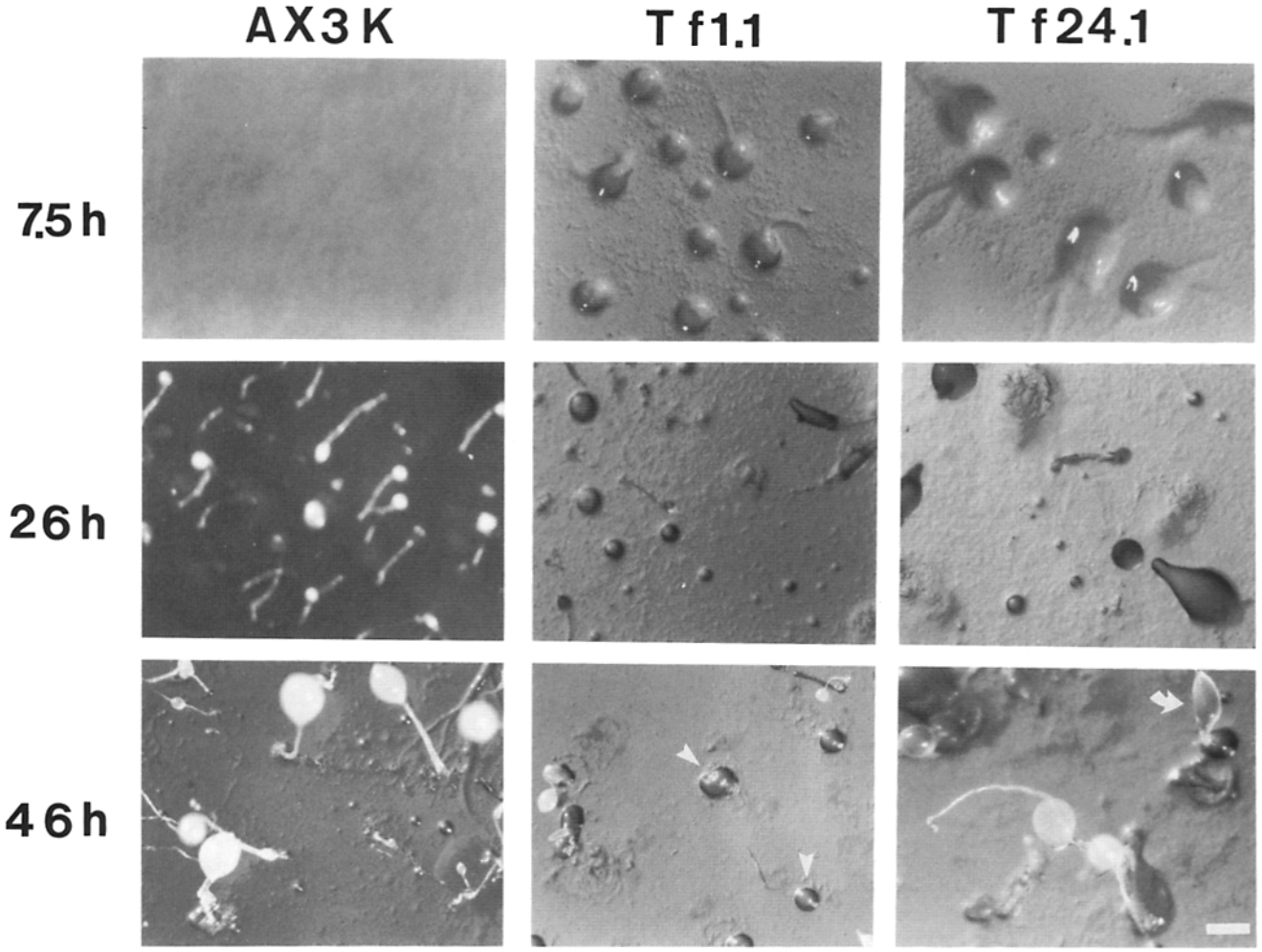


*Figure 11.* Ponticulin-minus cells initially develop faster, but development becomes asynchronous after aggregation into mounds. Representative fields of cells at 7.5 h, 26 h, and 46 h after initiation of development at 21°C. Bar, 0.5 mm. *Arrowhead*, mound; *arrow*, pseudoplasmodium.

unroofed ponticulin-minus cells contain greatly reduced amounts of adherent actin filaments (Figs. 7 and 8; Table II), indicating that ponticulin constitutes a major point of attachment between the plasma membrane and the cortical actin network in vivo as well as in vitro. Surprisingly, despite the loss of their major membrane-cytoskeleton attachment, the ponticulin-minus cells are essentially unimpaired for vegetative growth. These cells divide in suspension culture (Fig. 9), pinocytose FITC-dextran (Fig. 10 *A*), and phagocytose fluorescent beads in growth medium (Fig. 10 *B*) with kinetics indistinguishable from those of the parental cells. However, ponticulin-minus cells exhibit abnormalities (Fig. 11) that suggest a role for ponticulin in both early and late stages of multicellular development.

Based on actin-binding and nucleation assays and on the amount of cosedimenting endogenous actin, we estimate that the plasma membranes of cells lacking ponticulin have lost at least ~90% of the normal number of high affinity sites for actin. This estimate agrees well with previous conclusions from competition experiments with myosin subfragment-1 (26) and univalent fragments of R67 IgG (69) in which the ponticulin-mediated actin-binding activity was estimated to account for ~95% of the total actin-membrane binding. Although about 90% of the total membrane-associated actin also was lost from the surfaces of Tf24.1 cells in unroofing experiments (Table II; Fig. 8, *upper panel*), much of this loss was attributable to the decreased average filament length. Since the loss in filament number for both ponticulin-minus cell lines appeared to be only ~70%, as many as 30% of the total membrane-associated actin filaments in wild-type cells may be bound through low affinity interactions or by proteins that are lost or proteolyzed during membrane purification.

One interpretation of our observations is that many actin filaments are bound to the membrane through a low affinity, or labile, interaction involving one end of the filament, presumably the fast polymerizing end, as well as through multiple lateral interactions with ponticulin (Fig. 12). This interpretation is consistent with the decrease in polymerization lag time, relative to actin alone, that is sometimes observed in the presence of plasma membranes from ponticulin-minus cells (Fig. 6). This interpretation also is consistent with the observation that plasma membranes purified from cells containing ponticulin bind with high avidity to a constant mass of gelsolin-capped actin filaments over a wide range of filament lengths (56), whereas a constant, lower number of filaments are bound on rapidly isolated membranes from cells lacking ponticulin (Table II). Weak actin–membrane binding affinities in the ponticulin-minus cells also are consistent

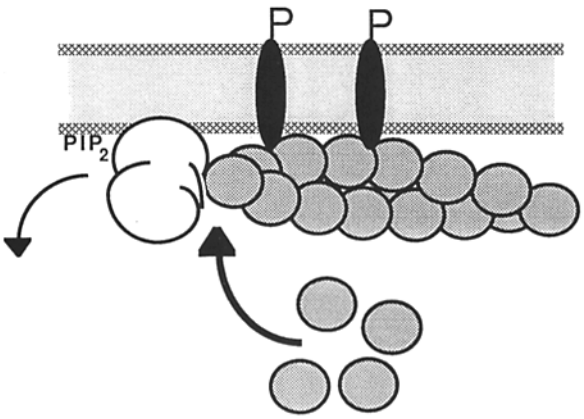


*Figure 12.* A diagrammatic model showing an actin filament attached to a membrane by two classes of actin-binding proteins. The first class of membrane-associated actin-binding proteins, typified by ponticulin (*P*) in *Dictyostelium*, mediates lateral nucleation of actin filaments and binds tightly to one or more sites along the sides of the filaments. The second class of membrane-associated actin-binding proteins is hypothesized to bind to one end of the actin filament, presumably the barbed end, with a much lower affinity. Dissociation of such a low affinity, and/or labile, interaction by regulatory factors at the membrane surface would permit elongation from this end of the filament.

with the even larger reduction in membrane-associated filaments observed after sedimentation through sucrose (Table I). Two such classes of membrane-binding sites could allow membrane-associated actin filaments to remain tightly bound to the membrane during elongation from their fast polymerizing ends, a process thought to be regulated by, and thus in the vicinity of, membrane polyphosphoinositides (recently reviewed in reference 10).

The near complete loss of high affinity–actin binding and nucleating activities from ponticulin-minus plasma membranes makes it extremely unlikely that another integral membrane protein is substituting for ponticulin function. Further, the sparseness of actin filaments associated with the cytoplasmic surfaces of ponticulin-minus cells strongly suggests that no other protein provides a comparable number of stable, high affinity links to the membrane in vivo. For instance, since large actin meshworks are retained on the cytoskeletal surfaces of erythrocytes and platelets (28) under conditions similar to those employed here (Fig. 8; Table II), the implication is that *Dictyostelium* amoebae lack significant numbers of spectrin- or filamin-mediated actin-membrane linkages.

One possible interpretation of our results is that the proposed labile and/or lower affinity links between the plasma membrane and the cortex somehow compensate for the absence of ponticulin. Alternatively, a stable physical connection between the plasma membrane and the cortex may not be required for cell viability and motility under laboratory conditions. Such a hypothesis is consistent with previous reports of pseudopod extension (61) and cytoplasmic streaming (1) in demembranated cytoplasm. Thus, cell motility, per se, may be primarily a function of the dynamic cortical cytoskeleton.

The relatively mild cellular phenotype of ponticulin-minus cells is reminiscent of the fairly subtle defects that have been reported for genetic knock-outs of other cytoskeletal proteins in *Dictyostelium*. For example, although quantitative deficiencies have been documented under certain circumstances, amoebae lacking ABP-120 (4, 13), alpha-actinin (67), annexin VII (synexin) (19), coronin (16), myosin I (34, 62, 66), myosin II (17, 45, 52), or severin (2) can grow and chemotax. Even double mutants lacking both alpha-actinin and ABP-120 are apparently unaltered in growth, chemotaxis, phagocytosis, and pinocytosis (68).

A relatively subtle cellular phenotype for ponticulin-minus cells also is consistent with the roles played by other membrane skeletal proteins in genetic diseases. For example, both protein 4.1 in hereditary spherocytosis (49) and dystrophin in muscular dystrophy (32) are typified by relatively mild cellular phenotypes that nevertheless affect long-term competitiveness and survival. Perhaps actin attachments to the plasma membrane should be considered as just one component of the total cell cytoskeleton, a structure formed by multiple components in an interlocking web of interactions (33). Loss, or diminution, of a single type of interaction may weaken the overall cytoskeletal framework but not be sufficiently deleterious to generate an obvious cellular defect.

Improper functioning of cytoskeletal components, however, may be generally more deleterious at the level of the organism. The uncoordinated morphogenesis of mound-stage ponticulin-minus cells leads to developmental inefficiencies that would probably result in extinction in the wild (Fig. 11). Other *Dictyostelium* cytoskeletal mutants have been described that also exhibit relatively mild vegetative phenotypes but severe defects in multicellular development. For instance, amoebae lacking functional myosin II (17, 20, 37, 45, 52) and cells deficient in both alpha-actinin and ABP-120 (68) become blocked at the mound stage of development. In conjunction with our findings (Fig. 11), these results suggest that proper cytoskeletal functioning and coordination are essential for normal morphogenesis in *Dictyostelium*.

Perhaps the most novel morphogenetic difference observed for the ponticulin-minus cells is the increased rate at which these cells aggregate into mounds (Fig. 11). This observation is consistent with a number of different molecular explanations. For instance, if disassociation of the tight ponticulin-mediated linkages between the membrane and the cortex is a prerequisite for pseudopod extension, ponticulin-minus cells might chemotax even faster than wild-type cells. Alternatively, a defective membrane-cytoskeleton interface could lead to abnormalities in membrane stability, membrane protein turnover, cortical structure, cell adhesion, and/or localization and functioning of signal transduction proteins. Finally, any of these primary defects could lead to secondary defects in ATP usage and energy stores that might accelerate the onset of development. These questions, among others, will be resolved through further analyses of membrane skeleton architecture and functioning in the ponticulin-minus cells.

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### *References*

1. Allen, R. D., J. W. Cooledge, and P. J. Hall. 1960. Streaming in cytoplasm dissociated from the giant amoeba, *Chaos chaos. Nature (Lond.).* 187:896–899.
2. André, E., M. Brink, G. Gerisch, G. Isenberg, A. Noegel, M. Schleicher, J. E. Segall, and E. Wallraff. 1989. A *Dictyostelium* mutant deficient in severin, an F-actin fragmenting protein, shows normal motility and chemotaxis. *J. Cell Biol.* 108:985–995.
3. Bennett, H., and J. Condeelis. 1988. Isolation of an immunoreactive analogue of brain fodrin that is associated with the cell cortex of *Dictyostelium* amoebae. *Cell Motil. Cytoskeleton.* 11:303–317.
4. Brink, M., G. Gerisch, G. Isenberg, A. A. Noegel, J. E. Segall, E. Wallraff, and M. Schleicher. 1990. A *Dictyostelium* mutant lacking an F-actin cross-linking protein, the 120-kD gelation factor. *J. Cell Biol.* 111:1477–1489.
5. Brown, S. S. 1993. Phenotypes of cytoskeletal mutants. *Curr. Opin. Cell Biol.* 5:129–134.
6. Caterina, M. J., and P. N. Devreotes. 1991. Molecular insights into eukaryotic chemotaxis. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 5:3078–3085.
7. Chia, C. P., and E. J. Luna. 1989. Phagocytosis in *Dictyostelium discoideum* is inhibited by antibodies directed primarily against common carbohydrate epitopes of a major cell-surface plasma membrane glycoprotein. *Exp. Cell Res.* 181:11–26.
8. Chia, C. P., A. L. Hitt, and E. J. Luna. 1991. Direct binding of F-actin to ponticulin, an integral plasma membrane glycoprotein. *Cell Motil. Cytoskeleton.* 18:164–179.
9. Chia, C. P., A. Shariff, S. A. Savage, and E. J. Luna. 1993. The integral membrane protein, ponticulin, acts as a monomer in nucleating actin assembly. *J. Cell Biol.* 120:909–922.
10. Condeelis, J. 1993. Life at the leading edge: the formation of cell protrusions. *Annu. Rev. Cell. Biol.* 9:411–444.
11. Condeelis, J., S. Ogihara, H. Bennett, J. Carboni, and A. Hall. 1987. Ultrastructural localization of cytoskeletal proteins in *Dictyostelium* amoebae. *Methods Cell Biol.* 28:191–207.
12. Cooper, J. A. 1991. The role of actin polymerization in cell motility. *Annu. Rev. Physiol.* 53:585–605.
13. Cox, D., J. Condeelis, D. Wessels, D. Soll, H. Kern, and D. A. Knecht. 1992. Targeted disruption of the ABP-120 gene leads to cells with altered motility. *J. Cell Biol.* 116:943–955.
14. Cramer, L. P., T. J. Mitchison, and J. A. Theriot. 1994. Actin-dependent motile forces and cell motility. *Curr. Opin. Cell Biol.* 6:82–86.
15. de Hostos, E. L., B. Bradtke, F. Lottspeich, R. Guggenheim, and G. Gerisch. 1991. Coronin, an actin binding protein of *Dictyostelium discoideum* localized to cell surface projections, has sequence similarities to G protein $\beta$ subunits. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:4097–4104.
16. de Hostos, E. L., C. Rehfueß, B. Bradtke, D. R. Waddell, R. Albrecht, J. Murphy, and G. Gerisch. 1993. *Dictyostelium* mutants lacking the cytoskeletal protein coronin are defective in cytokinesis and cell motility. *J. Cell Biol.* 120:163–173.
17. De Lozanne, A., and J. A. Spudich. 1987. Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination. *Science (Wash. DC).* 236:1086–1091.
18. Doonan, J. H. 1992. Cell division in *Aspergillus. J. Cell Sci.* 103:599–611.
19. Döring, V., M. Schleicher, and A. A. Noegel. 1991. *Dictyostelium* annexin VII (synexin). cDNA sequence and isolation of a gene disruption mutant. *J. Biol. Chem.* 266:17509–17515.
20. Eliott, S., G. H. Joss, A. Spudich, and K. L. Williams. 1993. Patterns in *Dictyostelium discoideum*: the role of myosin II in the transition from the unicellular to the multicellular phase. *J. Cell Sci.* 104:457–466.
21. Endow, S. A., and M. G. Titus. 1992. Genetic approaches to molecular motors. *Annu. Rev. Cell Biol.* 8:29–66.
22. Fukui, Y. 1993. Toward a new concept of cell motility: cytoskeletal dynamics in amoeboid movement and cell division. *Int. Rev. Cytol.* 144:85–127.
23. Fukui, Y., T. J. Lynch, H. Brzeska, and E. D. Korn. 1989. Myosin I is located at the leading edges of locomoting *Dictyostelium* amoebae. *Nature (Lond.).* 341:328–331.
24. Furukawa, R., S. Butz, E. Fleischmann, and M Fechheimer. 1992. The *Dictyostelium discoideum* 30,000 dalton protein contributes to phagocytosis. *Protoplasma.* 169:18–27.
25. Gerisch, G., A. A. Noegel, and M. Schleicher. 1991. Genetic alteration of proteins in actin-based motility systems. *Annu. Rev. Physiol.* 53: 607–628.
26. Goodloe-Holland, C. M., and E. J. Luna. 1984. A membrane cytoskeleton from *Dictyostelium discoideum.* III. Plasma membrane vesicles bind predominantly to the sides of actin filaments. *J. Cell Biol.* 99:71–78.
27. Goodloe-Holland, C. M., and E. J. Luna. 1987. Purification and characterization of *Dictyostelium discoideum* plasma membranes. *Methods Cell Biol.* 28:103–128.
28. Hartwig, J. H., and M. DeSisto. 1991. The cytoskeleton of the resting human blood platelet: structure of the membrane skeleton and its attachment to actin filaments. *J. Cell Biol.* 112:407–425.
29. Hartwig, J. H., K. A. Chambers, and T. P. Stossel. 1989. Association of gelsolin with actin filaments and cell membranes of macrophages and platelets. *J. Cell Biol.* 108:467–479.
30. Heuser, J., Q. Zhu, and M. Clarke. 1993. Proton pumps populate the contractile vacuoles of *Dictyostelium* amoebae. *J. Cell Biol.* 121:1311–1327.
31. Hitt, A. L., T. H. Lu, and E. J. Luna. 1994. Ponticulin is an atypical membrane protein. *J. Cell Biol.* 126:1421–1431.
32. Hoffman, E. P., and L. M. Kunkel. 1989. Dystrophin abnormalities in Duchenne/Becker muscular dystrophy. *Neuron.* 2:1019–1029.
33. Ingber, D. E. 1993. Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. *J. Cell Sci.* 104:613–627.
34. Jung, G., and J. A. Hammer III. 1990. Generation and characterization of *Dictyostelium* cells deficient in a myosin I heavy chain isoform. *J. Cell Biol.* 110:1955–1964.
35. Jung, G., Y. Fukui, B. Martin, and J. A. Hammer III. 1993. Sequence, expression pattern, intracellular localization, and targeted disruption of the *Dictyostelium* myosin ID heavy chain isoform. *J. Biol. Chem.* 268:14981–14990.
36. Kiehart, D. P. 1991. Contractile and cytoskeletal proteins in *Drosophila* embryogenesis. *Curr. Top. Memb.* 38:79–97.
37. Knecht, D. A., and W. F. Loomis. 1988. Development consequences of the lack of myosin heavy chain in *Dictyostelium discoideum. Dev. Biol.* 128:178–184.
38. Knecht, D. A., J. Jung, and L. Matthews. 1990. Quantification of transformation efficiency using a new method for clonal growth and selection of axenic *Dictyostelium* cells. *Dev. Genet.* 11:403–409.
39. Loomis, W. F. 1982. The Development of *Dictyostelium discoideum.* Academic Press, New York. 551 pp.
40. Loomis, W. F. 1993. Lateral inhibition and pattern formation in *Dictyostelium. Curr. Top. Dev. Biol.* 28:1–46.
41. Luna, E. J., and J. S. Condeelis. 1990. Actin-associated proteins in *Dictyostelium discoideum. Dev. Genet.* 11:328–332.
42. Luna, E. J., C. M. Goodloe-Holland, and H. M. Ingalls. 1984. A membrane cytoskeleton from *Dictyostelium discoideum.* II. Integral proteins mediate the binding of plasma membranes to F-actin affinity beads. *J. Cell Biol.* 99:58–70.
43. Luna, E. J., L. J. Wuestehube, C. P. Chia, A. Shariff, A. L. Hitt, and H. M. Ingalls. 1990. Ponticulin, a developmentally-regulated plasma membrane glycoprotein, mediates actin binding and nucleation. *Dev. Genet.* 11:354–361.
44. Luna, E. J., L. J. Wuestehube, H. M. Ingalls, and C. P. Chia. 1990. The *Dictyostelium discoideum* plasma membrane: a model system for the study of actin-membrane interactions. *Adv. Cell Biol.* 3:1–33.
45. Manstein, D. J., M. A. Titus, A. De Lozanne, and J. A. Spudich. 1989. Gene replacement in *Dictyostelium:* generation of myosin null mutants. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:923–932.
46. North, M. J. 1983. Solute uptake by *Dictyostelium discoideum* and its inhibition. *J. Gen. Microbiol.* 129:1381–1386.
47. O'Halloran, T. J., and R. G. W. Anderson. 1992. Clathrin heavy chain is required for pinocytosis, the presence of large vacuoles, and development in *Dictyostelium. J. Cell Biol.* 118:1371–1377.
48. Oster, G. 1988. Biophysics of the leading lamella. *Cell Motil. Cytoskeleton.* 10:164–171.
49. Palek, J., and S. Lambert. 1990. Genetics of the red cell membrane skeleton. *Sem. Hematol.* 27:290–332.
50. Peskin, C. S., G. M. Odell, and G. F. Oster. 1993. Cellular motions and thermal fluctuations–the Brownian ratchet. *Biophys. J.* 65:316–324.
51. Podolski, J. L., and T. L. Steck. 1990. Length distribution of F-actin in *Dictyostelium discoideum. J. Biol. Chem.* 265:1312–1318.
52. Pollenz, R. S., T.-L. L. Chen, L. Trivinos-Lagos, and R. L. Chisholm. 1992. The *Dictyostelium* essential light chain is required for myosin function. *Cell.* 69:951–962.
53. Scheel, J., K. Ziegelbauer, T. Kupke, B. M. Humbel, A. A. Noegel, G. Gerisch, and M. Schleicher. 1989. Hisactophilin, a histidine-rich actin-binding protein from *Dictyostelium discoideum. J. Biol. Chem.* 264: 2832–2839.
54. Schejter, E. D., and E. Wieschaus. 1993. Functional elements of the cytoskeleton in the early *Drosophila* embryo. *Annu. Rev. Cell Biol.* 9:67–99.
55. Schleicher, M., and A. A. Noegel. 1992. Dynamics of the *Dictyostelium* cytoskeleton during chemotaxis. *New Biol.* 4:461–472.

56. Schwartz, M. A., and E. J. Luna. 1986. Binding and assembly of actin filaments by plasma membranes from *Dictyostelium discoideum*. *J. Cell Biol.* 102:2067–2075.
57. Shariff, A., and E. J. Luna. 1990. *Dictyostelium discoideum* plasma membranes contain an actin-nucleating activity that requires ponticulin, an integral membrane glycoprotein. *J. Cell Biol.* 110:681–692.
58. Sheetz, M. P., D. B. Wayne, and A. L. Pearlman. 1992. Extension of filopodia by motor-dependent actin assembly. *Cell Motil. Cytoskeleton.* 22:160–169.
59. Spudich, J. A. 1987. *Dictyostelium discoideum:* molecular approaches to cell biology. *Methods Cell Biol.* 28:1–516.
60. Stossel, T. P. 1993. On the crawling of animal cells. *Science (Wash. DC).* 260:1086–1094.
61. Taylor, D. L., J. S. Condeelis, P. L. Moore, and R. D. Allen. 1973. The contractile basis of amoeboid movement. I. The chemical control of motility in isolated cytoplasm. *J. Cell Biol.* 59:378–394.
62. Titus, M. A., D. Wessels, J. A. Spudich, and D. Soll. 1993. The unconventional myosin encoded by the *myo*A gene plays a role in *Dictyostelium* motility. *Mol. Biol. Cell.* 4:233–246.
63. Van Haastert, P. J. M., P. M. W. Janssens, and C. Erneux. 1991. Sensory transduction in eukaryotes. A comparison between *Dictyostelium* and vertebrate cells. *Eur. J. Biochem.* 195:289–303.
64. Vogel, G. 1987. Endocytosis and recognition mechanisms in *Dictyostelium discoideum*. *Methods Cell Biol.* 28:129–137.
65. Welch, M. D., D. A. Holtzman, and D. G. Drubin. 1994. The yeast actin cytoskeleton. *Curr. Opin. Cell Biol.* 6:110–119.
66. Wesseles, D., J. Murray, G. Jung, J. A. Hammer III, and D. R. Soll. 1991. Myosin IB null mutants of *Dictyostelium* exhibit abnormalities in motility. *Cell Motil. Cytoskeleton.* 20:301–315.
67. Witke, W., W. Nellen, and A. Noegel. 1987. Homologous recombination in the *Dictyostelium* α-actinin gene leads to an altered mRNA and lack of the protein. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:4143–4148.
68. Witke, W., M. Schleicher, and A. A. Noegel. 1992. Redundancy in the microfilament system: abnormal development of *Dictyostelium* cells lacking two F-actin cross-linking proteins. *Cell.* 68:53–62.
69. Wuestehube, L. J., and E. J. Luna. 1987. F-actin binds to the cytoplasmic surface of ponticulin, a 17-kD integral glycoprotein from *Dictyostelium discoideum*. *J. Cell Biol.* 105:1741–1751.
70. Zigmond, S. 1993. Recent quantitative studies of actin filament turnover during cell locomotion. *Cell Motil. Cytoskeleton.* 25:309–316.