Mechanism of Actin Polymerization in Cellular ATP Depletion*

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Cellular ATP depletion in diverse cell types results in the net conversion of monomeric G-actin to polymeric F-actin and is an important aspect of cellular injury in tissue ischemia. We propose that this conversion results from altering the ratio of ATP-G-actin and ADP-G-actin, causing a net decrease in the concentration of thymosin-actin complexes as a consequence of the differential affinity of thymosin $\beta_4$ for ATP- and ADP-G-actin. To test this hypothesis we examined the effect of ATP depletion induced by antimycin A and substrate depletion on actin polymerization, the nucleotide state of the monomer pool, and the association of actin monomers with thymosin and profilin in the kidney epithelial cell line LLC-PK$_1$. ATP depletion for 30 min increased F-actin content to 145% of the levels under physiological conditions, accompanied by a corresponding decrease in G-actin content. Cytochalasin D treatment did not reduce F-actin formation during ATP depletion, indicating that it was predominantly not because of barbed end monomer addition. ATP-G-actin levels decreased rapidly during depletion, but there was no change in the concentration of ADP-G-actin monomers. The decrease in ATP-G-actin levels could be accounted for by dissociation of the thymosin-G-actin binary complex, resulting in a rise in the concentration of free thymosin $\beta_4$ from 4 to 11 $\mu$M. Increased detection of profilin-actin complexes during depletion indicated that profilin may participate in catalyzing nucleotide exchange during depletion. This mechanism provides a biochemical basis for the accumulation of F-actin aggregates in ischemic cells.

Recent progress in understanding the function of actin-binding proteins has clarified their role in a number of processes in normal cells, including motility and the establishment of cell polarity (1, 2). However, our understanding of actin dynamics and the roles of actin-binding proteins under conditions of cellular stress pertinent to pathophysiology is currently limited (3). For example, in tissue ischemia the lack of oxygen and nutrients is known to rapidly result in decreased intracellular ATP levels and increased ADP levels, varying in degree with the severity and duration of ischemic time (4, 5). These results have been associated with a concomitant decrease in cellular G-actin and a corresponding increase in the fraction of polymerized actin observed both in vivo and in vitro (6–11), with the resultant F-actin accumulating as dispersed aggregates throughout the cytoplasm (12, 13), notably in the perinuclear region (14–16).

Cells maintain a high potential energy for actin polymerization by maintaining a high total actin concentration and reserving a large fraction of this actin in a pool of monomers available for polymerization. This is of considerable functional importance for the cell, because the rate of actin polymerization is directly proportional to the local free monomer concentration (17). However, this high concentration requires the deployment of actin-binding proteins to maintain the monomer pool because, in the absence of other factors, all actin in excess of the dissociation equilibrium constant for subunit binding (commonly termed the critical concentration) polymerizes.

The high concentration of actin monomer is maintained by the activity of actin monomer-binding proteins (18). In most metazoan cells a large fraction of the monomer pool is probably associated with thymosins (T) (19). These low molecular weight (5,000) proteins act as monomer-sequestering factors in which thymosin-bound ATP-G-actin is unable to associate with filament ends or other monomers. The predominant forms of thymosin are thymosin $\beta_4$ and, to a much lesser extent, thymosin $\beta_1$. In contrast, profilin-bound monomers are able to associate with actin filament barbed ends (fast growing ends; the predominant site for actin polymerization in the cell), but association with filament pointed ends and spontaneous nucleation, or de novo polymerization, is blocked (20, 21). In most vertebrate cell types the concentration of the various forms of thymosin far exceeds that of profilin so that under normal physiological conditions the bulk of the monomer pool is associated with thymosin (22). The association and dissociation rates for the interaction between thymosin and actin are rapid and result in a very small pool of free G-actin (concentration <1 $\mu$M) and rapid flux of monomers between the thymosin-bound pool and F-actin (23).

Actin monomers bind either ATP or ADP, and both forms of G-actin are competent for polymerization. However, the nature of the bound nucleotide modulates the kinetics of association and dissociation, with distinct effects at the barbed and pointed ends of the filament (24). Moreover, the affinities of ATP- and ADP-G-actin monomers for thymosin and profilin differ considerably. Both profilin and thymosin $\beta_4$ bind ATP monomers with higher affinity than ADP monomers (21, 23), but the difference in affinity is greater by far for thymosin ($K_{D,ADP}$, 100 $\mu$M, $K_{D,ATP}$, 0.6 $\mu$M) (23). In normal cells this preference for ATP monomers ensures that the pool of unpolymerized actin consists almost entirely of ATP-G-actin with no significant amount of ADP-G-actin in the thymosin-sequestered pool (25, 26).

Conceivably, conditions such as ischemia that result in the depletion of ATP-G-actin and accumulation of ADP-G-actin would result in decreased levels of thymosin-bound actin as a direct result of the differential affinity of thymosin for ATP-

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1 The abbreviations used are: T, thymosin; TRITC, tetramethylrhodamine isothiocyanate.
ACTIN POLYMERIZATION AND ATP DEPLETION

A. Physiological Conditions

B. Cellular ATP Depletion

**FIG. 1.** Model showing the predominant flux of actin monomers under normal physiological conditions or conditions of ATP depletion. A, in the normal cell ATP predominates over ADP, and profilin-catalyzed nucleotide exchange results in accumulation of ATP-G-actin, which is rapidly bound by thymosin into a sequestered pool, $B$, when ATP is depleted the reversal of the normal ATP:ADP ratio results in accumulation of ADP-G-actin. The 100-fold lower affinity of thymosin for ADP monomers results in less G-actin being sequestered, increasing the concentration of polymerizable monomers above the critical concentration, resulting in F-actin formation.

and ADP-actin (Fig. 1B). The actin monomers thus released would increase the free monomer concentration to levels that exceed the critical concentration for polymerization, resulting in rapid polymerization at available nuclei or even, perhaps, initiation of new filament growth.

To directly test this hypothesis, which predicts that ATP depletion will result in decreased concentrations of ATP-actin monomers and increased free thymosin $\beta_4$, we measured the concentrations of ATP, ADP, ATP-actin, ADP-actin, and thymosin-actin using the mitochondrial poison antimycin A and substrate depletion to induce ATP depletion and mimic ischemia in LLC-PK$_1$ cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—A clonal line of LLC-PK$_1$ cells was grown to confluence in Dulbecco's modified Eagle's medium with 10% fetal bovine serum containing penicillin/streptomycin, passed once per week with 0.5% trypsin/EDTA, and seeded at a 1:8 dilution. Cultures typically reached confluence in 60- or 100-mm plates. Chemicals and culture media were purchased from Sigma unless otherwise noted.

**ATP Depletion**—Cells were ATP-depleted by incubating in depleted media (Dulbecco's modified Eagle's medium without glucose, pyruvate, or amino acids) containing 0.1% antimycin A for up to 30 min as previously described (27). For experiments measuring the effect of variable cellular levels of ATP on actin polymerization, cells were depleted in depletion media containing 0–1000 ng/ml/culture buffer. In some experiments, cells were incubated in 0.1 M cytosol D for 30 min before depletion and during cellular ATP depletion.

**Cellular G-actin Isolation in Triton X-100-soluble Protein Extracts**—Cells were extracted with 200 μl of Triton extraction buffer (0.1% Triton X-100 in PHEM (60 mM 1,4-piperazinediethanesulfonic acid, 25 mM HEPES, 10 mM EGTA, and 2 mM MgSO$_4$) with CLAP (10 μg/ml each chymostatin, leupeptin, aprolin, and pepstatin), and 0.5 mM phenylmethylsulfonyl fluoride) with 20 μM latrunculin A (Molecular Probes, Eugene, OR), and 0.1 mg/ml phallolidin.

Two 100-μl aliquots per plate of supernatant were spun through PHEM buffer-equilibrated P-6 spin columns (Bio-Rad) to remove free nucleotides at 2000 × g for 5 min at 4 °C. To isolate actin with its bound nucleotides, we used immobilized DNase I (28).

**Actin Polymerization and ATP Depletion**

**RESULTS**

**ATP Depletion Results in Net Conversion of G- to F-Ac tin**—We used ATP depletion in a clonal line of LLC-PK$_1$ cells as a model of ischemic injury (33). Cells were treated with the mitochondrial cytochrome bc$_1$ inhibitor antimycin A (0.1 μM) and with substrate-depleted medium to eliminate both oxidative phosphorylation and glycolysis as sources of ATP. The intracellular ATP concentration fell rapidly in response to this treatment (Fig. 2A), from control levels of 1.7 to 0.03 μM after 15 min of depletion. The concentration of ADP rose to a peak of 0.55 μM at 5 min of depletion, decreasing to 0.2 μM at 30 min. The ratio of ATP:ADP fell from 93 to 25% by 15 min (Fig. 2A) and decreased further to 15% by 30 min of ATP depletion.
To quantitatively assess the effect of depleting intracellular ATP on the actin cytoskeleton, we measured the fraction of filamentous and monomeric actin as a function of the duration of ATP depletion (Fig. 2B). Total cellular F-actin was determined by quantifying the binding of fluorescent phalloidin (a fungal alkaloid that specifically binds filamentous but not monomeric actin) to fixed, permeabilized cells, and G-actin was quantified based on its ability to inhibit the hydrolitic activity of DNase I. With ATP depletion times of up to 30 min there was a roughly linear increase in the filamentous fraction of actin, to a maximum level 45% higher than that observed under physiological conditions (33). Independent measurements documented a decrease in the monomeric fraction of actin, to 50% below the level under physiological conditions, consistent with the increase in F-actin. The total intracellular actin concentration measured by immunoblotting was 27 \( \mu \text{M} \), whereas the baseline G-actin concentration was 16 \( \mu \text{M} \) (Table I). Therefore, the alterations observed in G- and F-actin levels represent a decrease in monomeric actin from 16 to 8 \( \mu \text{M} \), with a corresponding increase in F-actin from \( \sim 11 \) to 19 \( \mu \text{M} \); values that are consistent within the error in the data. Continued ATP depletion beyond 30 min did not result in further significant increases in the F-actin content, which remained at 30–40% above baseline after 60 min of ATP depletion. We also measured the extent of F-actin incorporation in a Triton X-100-insoluble fraction using high speed centrifugation (48,000 \( \times g \)) to pellet large filaments or filaments cross-linked into the bulk cytoskeleton (Fig. 2C). The additional F-actin formed after 30 and 60 min of depletion was incorporated into the Triton-insoluble material that was recovered in the high speed pellet. No significant additional incorporation into small oligomeric filaments (437,000 \( \times g \)) was observed (data not shown). Actin polymerization triggered by ATP depletion was not the result of additional synthesis of actin monomers, as cycloheximide (10 \( \mu \text{g/ml} \)) treatment had no effect on F-actin levels during depletion or recovery (data not shown). As previously shown in our studies and by other investigators (33), ATP-depleted cells lost microvillar actin bundles and stress fibers and, instead, accumulated phalloidin-staining aggregates throughout the cytoplasm, particularly in the perinuclear area (not shown).

To further explore the nature of the process leading to F-actin formation in ATP-depleted cells, we incubated cells with the fungal toxin cytochalasin D (0.1 \( \mu \text{M} \)) to inhibit actin filament elongation by monomer addition onto filament barbed ends (Fig. 2D) (34). Surprisingly, cytochalasin D had little effect on F-actin accumulation during ATP depletion and, in fact, resulted in significantly increased F-actin accumulation after 15 min of ATP depletion (\( p < 0.01 \)).

We next sought to determine the energy level below which actin polymerization was initiated. To accomplish this we added back a range of glucose concentrations to the medium during 30 min of antimycin A treatment (Fig. 3). In the presence of antimycin A the intracellular ATP:ADP ratio varied linearly as a function of the glucose concentration over a range of glucose concentrations from 0 to 60 mg/liter. At glucose concentrations greater than 40 mg/ml, in which the ATP:ADP ratio was greater than 0.31, there was no effect on F-actin levels, but at lower glucose concentrations, in which the ATP:ADP ratio fell below 0.3 (intracellular ATP and ADP concentrations of 22 \( \mu \text{M} \) and 72 \( \mu \text{M} \), respectively), a progressive increase in F-actin content was observed. The F-actin content was a linear function of the decrease in the ATP:ADP ratio below 0.3 as the glucose concentration was decreased from 40 to 1 mg/liter (ATP concentrations are 22–15 \( \mu \text{M} \), with ADP concentrations remaining roughly constant in the range of 65–75 \( \mu \text{M} \)).

**Effect of ATP Depletion on the Nucleotide Bound to the Monomer Pool**—To determine the effect of ATP depletion on the nucleotide state of G-actin, we isolated unpolymerized G-actin monomers by binding them to DNase I immobilized on beads and measured the fraction of ATP and ADP associated with the monomers (Fig. 4). We included latrunculin B and phalloidin in the isolation buffer to inhibit nucleotide exchange (35) and polymerization or depolymerization during the isolation procedure. As previously noted, the cellular concentration of G-actin declined with ATP depletion, from 16 to 5 \( \mu \text{M} \) in control cells after 15 min of ATP depletion (Fig. 4). There was a corresponding decrease in the concentration of ATP G-actin. However, the concentration of ADP G-actin remained essentially constant at approximately 0.5 \( \mu \text{M} \) throughout the time course.
Intracellular concentration | Percent soluble protein
--- | ---
Total actin | 27.9 ± 6.8 | ND
G-actin | 15.8 ± 7.2 | 7.6 ± 1.3
Thymosin β4 | 11.1 ± 3.8 | 1.24 ± 0.1
Profilin | 2.0 ± 0.4 | 0.38 ± 0.07

### Role of Monomer-binding Proteins

To evaluate the potential importance of different classes of actin monomer-binding proteins, we measured their concentrations in the LLC-PK1 cells used for these experiments (Table I). Thymosin β4 was present at high concentrations (26 ± 8.8 μM), approaching the concentration of G-actin in the cells under normal growth conditions (31 ± 7.2 μM). The concentration of profilin was much lower (2.0 ± 0.4 μM). As a comparison we measured the levels of these proteins as a fraction of the total soluble protein in LLC-PK1 cells and in cortical tissue (primarily proximal tubule epithelial cells) isolated from rat kidney. Levels in isolated tubules were very similar to those found in the LLC-PK1 cells (Table I), confirming the physiological significance of our results for ischemic injury in the kidney.

To quantitatively analyze the effect of ATP depletion on G-actin association with sequestering proteins, we used filters with a molecular weight cutoff of 10,000 to separate the free fraction of thymosin β4 from the fraction associated with G-actin (Fig. 5A). In cells under normal growth conditions the concentration of free thymosin β4 was ~4 μM, rising to 11 μM after 15 min of ATP depletion (Fig. 5B). This corresponds to a release of all thymosin-bound actin monomers in the first 15 min of ATP depletion. We next compared the relative abundance of thymosin-actin complexes by native gel electrophoresis, using immunoblotting with anti-actin antibodies to confirm the identity of the complexes observed (Fig. 5C). On native gels, thymosin-actin complexes migrate slightly ahead of free actin monomers, probably because thymosin binding restricts the repertoire of conformations available to actin (Fig. 5C, arrow) (26, 36). On native gels of control cell extracts G-actin migrated predominantly in the fast migrating, thymosin-bound fraction (Fig. 5C, lane 1, arrow), whereas in extracts from cells subjected to 15 min of ATP depletion less G-actin was detected, and the electrophoretic mobility profile was characteristic of free actin monomers (Fig. 5C, lane 2).
We also used native gel electrophoresis (31) to analyze the extent of actin monomer association with profilin (Fig. 5D). Immunoblotting showed increased levels of profilin co-migrating with actin monomer in extracts from cells depleted for 15 min (compare Fig. 5D, lanes 4 and 5) that was reversed by the addition of an ATP regenerating system to the extract (lane 6).

**DISCUSSION**

In this report we show evidence for a direct causal mechanism for unregulated actin polymerization based on the differential affinity of ATP- and ADP-actin monomers for the highly abundant monomer sequestering protein thymosin \( \beta_4 \). We confirmed that ATP depletion induced by substrate depletion and antimycin A resulted in net conversion of G-actin to F-actin. The increase in the fraction of cellular actin recovered by centrifugation or labeled as polymer by phalloidin binding was essentially complete in the first 15 min of depletion. Experiments in which we manipulated the ATP:ADP ratio by incubating the cells in limiting concentrations of glucose point to a mechanism that directly involves the ATP concentration or the ATP:ADP ratio because they showed a critical threshold ATP:ADP ratio below which there was an inverse linear relationship.

Maintenance of a high concentration of available monomers at the critical concentrations do not differ appreciably between the pointed and barbed ends. Not all eukaryotic cell types express high concentrations of thymosin filament barbed ends. Not all eukaryotic cell types express high concentrations of thymosin

ADP/actin formation during ATP depletion was not inhibited by treatment with cytochalasin D, and in fact, we observed that cytochalasin-treated cells accumulated significantly more F-actin than in the absence of the drug. At the concentration used here (0.1 \( \mu M \)) cytochalasin D binds actin monomers and incompletely inhibits the addition of monomers onto the barbed ends of actin filaments (34), with more potent inhibition of the addition of ATP-actin than of ADP-actin. Our results using cytochalasin imply that much of the F-actin formed during ATP depletion does not arise by barbed end polymerization (the mechanism that accounts for the bulk of actin polymerization induced by normal stimuli) but rather by pointed end addition, which is inhibited to a much lesser degree by cytochalasin treatment. As pointed out by Sampath and Pollard (38), the conversion of ATP- to ADP-actin may be accelerated by cytochalasin treatment, which could account for the significantly higher levels of F-actin in depleted cells treated with cytochalasin compared with those in the absence of the drug. The dissipation of this effect at later time points supports a kinetic interpretation of this effect.

These results suggest that the mechanism of F-actin formation in ATP-depleted cells is markedly different than that which prevails when ATP predominates over ADP as it does in normal healthy cells, in which case pointed end polymerization is insignificant for several reasons: 1) the critical concentration for polymerization of ATP monomers is 20-fold lower at the barbed end than at the pointed end; 2) monomers bound to profilin are able to add onto filament barbed ends but not onto pointed ends or to participate in de novo filament formation; and 3) the pointed ends of most actin filaments in the cytoskeleton of normal cells are inaccessible for monomer addition because most are capped (2). However, in the conditions that prevail during ischemia or in model ATP depletion, assuming that most actin monomers are in the ADP-bound form, the critical concentrations do not differ appreciably between the pointed and barbed ends (24), and monomers will more readily dissociate from profilin because of the 5–8-fold lower affinity of profilin for ADP monomers (21) and thus be available for pointed end addition.

Our model (Fig. 1) makes the following predictions. 1) The concentration of thymosin \( \beta_4 \) is high enough in these cells to sequester a substantial fraction of the unpolymerized ATP-G-actin. We measured a concentration of thymosin \( \beta_4 \) in LLC-PK \(_4\) cells of 11 \( \mu M \), a value that lies within the error for the measured concentration of G-actin in these cells at base line. 2) ATP depletion results in a decrease in the concentration of ATP monomers but not ADP monomers. We measured a steep decline in ATP-G-actin concentration but little alteration in ADP-G-actin concentration during depletion. Indeed, the ratio of ATP:ADP monomers after 30 min of depletion (Fig. 4, roughly 5:1) is much higher than would be expected from the 1.7 ratio of ATP:ADP in the cytosol at this time point (which, with the 3-fold higher affinity of monomer for ATP, would predict a 1:2 ratio of ATP:ADP monomer), consistent with flux of ADP monomers from the unpolymerized to the polymerized state. 3) The concentration of free (unbound) thymosin increases in proportion to the decrease in the concentration of the G-actin pool. 4) Increased levels of profilin-actin complexes could increase the rate of exchange of ADP for ATP on actin monomers. The data presented here support all aspects of this model.

ADF/cofilin proteins bind actin monomers (in addition to their effects on actin filaments) and are dephosphorylated and thereby activated during ATP depletion. The concentration of ADF in these LLC-PK \(_4\) cells is low (0.2 \( \mu M \); Ref. 39) but there are higher levels of cofilin present that we estimate to be equivalent to the levels of profilin. Although ADF/cofilin binds tightly to ADP monomers, profilin effectively competes for binding (21, 40) and would prevent sequestration of a significant fraction of these monomers in ATP-depleted cells. ADF/cofilin can also markedly increases the rate of polymerization of ATP-actin at both ends of actin filaments (41) and so could accelerate the rate of F-actin formation during depletion.

Release of ATP-actin monomers from thymosin \( \beta_4 \) occurs as part of the mechanism that drives rapid actin polymerization in the normal function of the cytoskeleton in cell morphology and cell motility (42). Thymosin maintains the bulk of the monomer pool in the cells of metazoans, thanks to its abundance and relatively high affinity for ATP monomers (22). Maintenance of a high concentration of available monomers at nuclei or filament barbed ends is necessary for rapid actin polymerization. The low affinity of the interaction between ADP monomers and thymosin is necessary to allow monomers to be handed on so that catalysis of nucleotide exchange by profilin regenerates ATP monomers for rapid addition onto filament barbed ends. Not all eukaryotic cell types express high concentrations of thymosin \( \beta_4 \) or equivalent sequestering proteins. In amoeba, for example, the concentration of profilin is sufficient, based on its affinity for actin, to account for the entire monomer pool, and thymosin-like factors do not seem to play a significant role (21, 43). It would perhaps be instructive to compare the effect of ATP depletion in cells such as these that operate in the absence of thymosin. It is interesting to note that in cell types that normally express significant quantities of thymosin its loss is associated with changes, including formation of actin clumps, that are remarkably congruent with the consequences of ischemia or ATP depletion (44).

It is tempting to rationalize the cytoskeletal changes we describe into some protective mechanism that has evolved to respond to an energy crisis in the cell. Indeed, it has been shown that inhibition of actin dynamics in neurons subjected to ATP depletion can result in reduced levels of cell death (44). However, because our mechanism seems to result directly from the fundamental properties of the system of actin and mono-
mer-binding proteins this may not be the case. It is notable that cells such as the free living protozoa and fungi that are exposed to direct selection in favor of surviving episodes of energy depletion are the very cell types that do not use thymosin as part of their actin regulation (2, 21, 43). Whatever advantage is conferred by the additional monomer buffering provided by thymosin may be outweighed by the disadvantageous effects of the release of a large fraction of the pool when the cell is stressed energetically. Further studies will be necessary to determine whether polymerization of monomers by the mechanism described here is directly toxic to cells.

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REFERENCES
1. Nelson, W. J. (2003) Nature 422, 766–774
2. Pollard, T. D., and Borisy, G. G. (2003) Cell 112, 453–465
3. Atkinson, S. J., and Molitoris, B. A. (2001) in Acute Renal Failure: A Companion to Brenner and Rector’s The Kidney (Molitoris, B. A., and Finn, W. F., eds) 1st Ed., W. B. Saunders, Philadelphia
4. Gerlach, E., Deuticke, B., Dreisbach, R., and Rosarious, C. (1983) Pflügers Arch. 278, 296–315
5. Weinberg, J. M. (1991) Kidney Int. 39, 476–500
6. Sutton, T. A., and Molitoris, B. A. (1998) Semin. Nephrol. 18, 490–497
7. Molitoris, B. A., Geerdes, A., and McIntosh, J. R. (1991) J. Clin. Invest. 88, 462–469
8. Kwon, O., Phillips, C. L., and Molitoris, B. A. (2002) Am. J. Physiol. 282, F1012–F1019
9. Jahraus, A., Egeberg, M., Hinner, B., Habermann, A., Sackman, E., Pralle, A., Faulstich, H., Rybin, V., Defauche, H., and Griffiths, G. (2001) Mol. Biol. Cell 12, 155–170
10. Hinshaw, D. B., Armstrong, B. C., Burger, J. M., Beals, T. F., and Hyslop, P. A. (1988) Am. J. Pathol. 132, 479–488
11. Hinshaw, D. B., Armstrong, B. C., Beals, T. F., and Hyslop, P. A. (1988) J. Surg. Res. 44, 527–537
12. Kellerman, P. S., Clark, R. A., Haulien, C. A., Linas, S. L., and Molitoris, B. A. (1990) Am. J. Physiol. 259, F279–F285
13. Kuhne, W., Besselmann, M., Noli, T., Muhs, A., Watanabe, H., and Piper, H. M. (1993) Am. J. Physiol. 264, H1559–H1608
14. Glascott, P. A., Jr., McSorley, K. M., Mittal, B., Sanger, J. M., and Sanger, J. W. (1987) Cell Motil. Cytoskeleton 8, 118–129
15. Shelden, E. A., Weinberg, J. M., Sorenson, D. R., Edwards, C. A., and Pollock, F. M. (2002) J. Am. Soc. Nephrol. 13, 2667–2680
16. Herget-Rosenthal, S., Haaford, M., Kribben, A., Atkinson, S. J., Sandoval, R. M., and Molitoris, B. A. (2001) Am. J. Physiol. 281, C1858–C1870
17. Cooper, J. A. (1991) Annu. Rev. Physiol. 53, 585–605
18. Sun, H. Q., Kwiatkowska, K., and Yin, H. L. (1995) Curr. Opin. Cell Biol. 7, 102–110
19. Nachmias, V. T. (1993) Curr. Opin. Cell Biol. 5, 56–62
20. Pollard, T. D., and Cooper, J. A. (1984) Biochemistry 23, 6631–6641
21. Vincen, V. K., De La Cruz, E. M., Higgs, H. N., and Pollard, T. D. (1998) Biochemistry 37, 10871–10880
22. Cassimeris, L., Safer, D., Nachmias, V. T., and Zigmond, S. H. (1992) J. Cell Biol. 119, 1261–1270
23. Carlier, M. F., Jean, C., Rieger, K. J., Lenfant, M., and Pantaloni, D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5034–5038
24. Pollard, T. D. (1986) J. Cell Biol. 103, 2747–2754
25. Safer, D., and Nachmias, V. T. (1994) BioEssays 16, 473–479
26. De La Cruz, E. M., Ostap, E. M., Brundage, R. A., Reddy, K. S., Sweeney, H. L., and Safer, D. (2000) Biophys. J. 78, 2516–2527
27. Molitoris, B. A., Dahl, R., and Hoaford, M. (1996) Am. J. Physiol. 271, F790–F798
28. Rosenblatt, J., Peluso, P., and Mitchisen, T. J. (1995) Mol. Biol. Cell 6, 227–236
29. Dagher, P. C. (2000) Am. J. Physiol. 279, C1270–C1277
30. Cano, M. L., Lauffenburger, D. A., and Zigmond, S. H. (1991) J. Physiol. 115, 677–687
31. Safer, D. (1989) Anal. Biochem. 178, 32–37
32. Weinberg, J. M. (1985) J. Clin. Invest. 76, 1193–1208
33. Canfield, P. E., Geerdes, A. M., and Molitoris, B. A. (1991) Am. J. Physiol. 261, F1038–F1045
34. Cooper, J. A. (1987) J. Cell Biol. 105, 1473–1478
35. Ayscough, K. R., Stryker, J., Pukala, N., Sanders, M., Crews, P., and Drumbl, D. G. (1997) J. Cell Biol. 137, 399–416
36. Safer, D., Golla, R., and Nachmias, V. T. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2536–2540
37. Venkataramaiah, M. A., Patel, Y. J., Kreisberg, J. I., and Weinberg, J. M. (1988) J. Clin. Invest. 81, 745–758
38. Sampath, P., and Pollard, T. D. (1991) Biochemistry 30, 1973–1980
39. Ashworth, S. L., Southgate, E. L., Sandoval, R. M., Meberg, P. J., Bamburg, J. R., and Molitoris, B. A. (2000) Am. J. Physiol. 280, F952–F962
40. Mockrin, S. C., and Korn, E. D. (1980) Biochemistry 19, 5359–5362
41. Blanchon, L., and Pollard, T. D. (1998) J. Biol. Chem. 273, 25106–25111
42. Pantaloni, D., and Carlier, M. F. (1993) Cell 75, 1007–1014
43. Kaiser, D. A., Vincen, V. K., Murphy, D. B., and Pollard, T. D. (1999) J. Cell Sci. 112, 3779–3790
44. Iguchi, K., Utsami, T., Hirano, K., Hamatake, M., Shibata, M., and Ishida, R. (1999) Biochem. Pharmacol. 57, 1105–1111