The highly related actin isoforms are thought to have different functions. We recently demonstrated a polarized distribution of actin isoforms in gastric parietal cells and association of gastric ezrin with the cytoplasmic β-actin isoform (Yao, X., Chaponnier, C., Gabbiani, G., and Forte, J. G. (1995) Mol. Biol. Cell. 6, 541-557). Here we used ultrastructural immunocytochemistry to verify that β-actin is located within canalicular microvilli and the apical cortex of parietal cells, similar to the localization reported for ezrin. Furthermore, we tested whether ezrin binds preferentially to cytoplasmic β-actin compared with the skeletal muscle α-actin isoform. Purified cytoplasmic β-actin (from erythrocytes) and skeletal α-actin were assembled with gastric ezrin. Co-sedimentation experiments showed that gastric ezrin selectively co-pelleted with the β-actin isoform and only very poorly with α-actin. Binding of erythrocitic β-actin to ezrin is saturable with a molar ratio of ~1:10 (ezrin:actin) and a dissociation constant ~4.6 × 10^{-8} M. In addition, ezrin promoted pyrene-labeled actin assembly, with predominant effects on filament elongation and a distinct preference for β-actin compared with α-actin. Given these isoform-selective associations, we speculate that actin isoforms might segregate into different functional domains and exert specificity by interacting with isoform-orientated binding proteins.

Although actin is a highly conserved protein, several distinct tissue-specific isoforms exist. The actin isoforms are encoded by separate genes and differ by less than 10% in amino acid sequence (1) and are generally believed to exert different functions. For example, profilin has different affinities for each of the cytoplasmic actin isoforms, β-actin and γ-actin, and for sarcomeric actin (2, 3). We showed recently that there is a polarized distribution of cytoplasmic β- and γ-actin isoforms in gastric parietal cells (4), consistent with some difference in functional activity and/or preferential interaction with localized actin-binding proteins. The β-actin isoform is concentrated near the apical membrane of all gastric epithelial cells, including the apical secretory canalicular membrane of parietal cells, while the γ-actin isoform is primarily distributed toward the basolateral surface, with minor deposition in the region of the secretory canaliculi of parietal cells.

Ezrin is an actin-binding protein of the ezrin/radixin/moesin (ERM) family of cytoskeleton-membrane linker proteins (5). Within the gastric epithelium, ezrin has been localized exclusively to the apical canalicular membrane of parietal cells (6, 7). Because of its cytolocalization and stimulation-dependent phosphorylation, an implied role for ezrin has been suggested in the apical surface membrane remodeling associated with parietal cell activation via the protein kinase A pathway (6, 8). Phosphorylation of ezrin has also been associated with surface membrane remodeling of A431 cells stimulated by epidermal growth factor (9), although activation in this case was via protein tyrosine kinase. Our previous studies showed that the F-actin which co-localized with ezrin in parietal cells was primarily comprised of the β-actin isoform, and that the β-actin isoform was preferentially co-immunoprecipitated with ezrin from extracts of gastric membranes (4). Recently, Shuster and Herman (10) reported that ezrin, contained within lysates of retinal pericytes, bound to immobilized matrices of cytoplasmic β-actin, but not to the skeletal α-actin isoform. These authors further demonstrated a co-localization of antibodies against the β-actin isoform and ezrin in leading lamellae of motile cells. Thus a body of evidence suggests interaction of ezrin with the actin cytoskeleton may be specific for the β-actin isoform, but much controversy remains regarding the nature of the interaction and whether it is direct or via intermediary proteins.

The purpose of the present experiments was to test if gastric ezrin exerts isoform-specific association with cytoplasmic β-actin isoforms in relatively simple reconstituted systems. Accordingly, we separately assembled skeletal α-actin and cytoplasmic β-actin in the presence of gastric ezrin and subjected them to centrifugation. Gastric ezrin selectively co-sedimented with cytoplasmic β-actin compared with skeletal α-actin, which is consistent with an earlier observation that showed a weak interaction between skeletal α-actin and intestinal ezrin (11), and supports the notion of isoform specificity. Further characterization of ezrin-actin interaction by using erythrocytic β-actin revealed that gastric ezrin binds to actin in a saturable manner with a molar ratio of about 1:10 (ezrin:actin) and a dissociation constant (K_d) ~4.6 × 10^{-8} M for the ezrin-actin binding relationship.

MATERIALS AND METHODS

Purification of Actin Isoforms—An acetone powder was prepared from rabbit muscle using the method of Pardee and Spudich (12). Typically, we obtained ~10 mg of pure skeletal actin from 1 g of acetone powder. Cytoplasmic β-actin was purified from bovine red blood cells as described by Pusekine et al. (13). Briefly, bovine red blood cells (RBCs) were osmotically hemolyzed, and an acetone powder was made from the harvested RBC ghosts. The resulting acetone powder was then extracted twice with 15 ml buffer A (5 mM Tris, pH 8.0, 0.2 mM CaCl_2, 0.2 mM ATP, 0.2 mM DTT, 0.02% sodium azide)/mg of acetone powder for 3 h at 4 °C. After clarification at 27,000 × g for 1 h at 4 °C, the extract was fractionated over a DE52 anion exchange column pre-equilibrated with buffer A, and developed with a linear gradient of 50 mM to 1 M NaCl, identified by dot-blot. These fractions were pooled, concentrated to 4 ml, and applied to a 2 × 40-cm Sephadex G-150 column equilibrated with buffer A. The peak actin fractions were then pooled in buffer A containing 2 mM sodium azide.

The abbreviations used are: RBC, red blood cells; DTT, dithiothreitol; MES, 4-morpholinethanesulfonic acid.
Purification of Ezrin—Gastric ezrin was purified from rabbit gastric mucosal homogenates using a procedure described by Breitscher (14) with slight modification. Gastric ezrin was extracted with buffer containing 0.5% Triton X-100, 300 mM NaCl, 1 mM EGTA, 0.25 mM phenylmethylsulfonyl fluoride, 10 μM E64, 1 mM benzamidine, 20 mM Tris-Cl, pH 7.4, with stirring on ice for 60 min to maximize the extraction of ezrin, and centrifuged at 20,000 × g for 20 min. The supernatant was brought to 40% (NH₄)₂SO₄ and the precipitate removed by centrifugation at 12,000 × g for 20 min. The resulting supernatant was precipitated with 75% (NH₄)₂SO₄. The precipitates were solubilized in 10 mM imidazole, pH 6.7, 1 mM DTT and dialyzed against the same buffer overnight. After clarification at 30,000 × g for 20 min, the supernatant was applied to a hydroxyapatite column pre-equilibrated with 100 mM potassium phosphate, pH 7.0, and developed with a 40-ml linear gradient of 100–800 mM potassium phosphate. Fractions containing ezrin were identified by dot-blot immunostaining of dialyzed fractions for ezrin by an antibody directed against the B-actin isoform within gastric glandular cells using immunoelectron microscopy. A micrograph shows a cross-section through a gastric gland including several parietal cells (PC) and mucous neck cells (MNC) surrounding the gland lumen. There is a dense distribution of enhanced gold particles along the apical surfaces (Ap) of all epithelial cells, especially within parietal cells along the intracellular canalicular (IC), which are invaginations of the apical surface membrane coursing throughout the parietal cell. Gold particles are also seen along the basolateral region (BI) of parietal cells, with a somewhat lower density than along canalicular. Large mitochondria throughout the cytoplasm are characteristic of parietal cells; numerous cytoplasmic tubulovesicles and canalicular microvilli, also characteristic of parietal cells, are not easily visualized at this magnification. Bar marker is 2 μm.

Coomassie Blue and dried between sheets of cellulose for visualization and quantitative scanning. Other were transblotted to nitrocellulose and probed with ezrin antibodies.Pyrene-labeled Actin Assembly—Pyrene-labeled actin was prepared by modifying the method of Kauyama and Mihashi (17). Briefly, 100 mg of polymerized actin was pelleted and homogenized in buffer P (10 mM Hepes, pH 7.4, 2 mM MgCl₂, 100 mM KCl, 0.5 mM ATP), 5.6 mg of N-(1-pyrenyl)iodoacetamide (Molecular Probes, Inc., Eugene, OR) was added with agitation, and the solution was incubated at 4°C in the dark overnight. The labeled actin was dialyzed against 100 mM 0.05% Tween 20, 0.05% Triton X-100, 300 mM NaCl, 1 mM EGTA, 0.25 mM phenylmethylsulfonyl fluoride, 10 μM E64, 1 mM benzamidine, 20 mM Tris-Cl, pH 7.4, with stirring on ice for 60 min to maximize the extraction of ezrin, and centrifuged at 80°C. Theyield of ezrin-enriched fractions was relatively poor, and quantitative scanning. Other were transblotted to nitrocellulose and probed with ezrin antibodies. Pyrene-labeled ezrin antibody was prepared by modifying the method of Kauyama and Mihashi (17). Briefly, 100 mg of polymerized ezrin was pelleted and homogenized in buffer P (10 mM Hepes, pH 7.4, 2 mM MgCl₂, 100 mM KCl, 0.5 mM ATP), 5.6 mg of N-(1-pyrenyl)iodoacetamide (Molecular Probes, Inc., Eugene, OR) was added with agitation, and the solution was incubated at 4°C in the dark overnight. The labeled ezrin was dialyzed against 100 mM 0.05% Tween 20, 0.05% Triton X-100, 300 mM NaCl, 1 mM EGTA, 0.25 mM phenylmethylsulfonyl fluoride, 10 μM E64, 1 mM benzamidine, 20 mM Tris-Cl, pH 7.4, with stirring on ice for 60 min to maximize the extraction of ezrin, and centrifuged at 80°C. Theyield of ezrin-enriched fractions was relatively poor, and quantitative scanning. Other were transblotted to nitrocellulose and probed with ezrin antibodies.

Coomassie Blue and dried between sheets of cellulose for visualization and quantitative scanning. Other were transblotted to nitrocellulose and probed with ezrin antibodies. Pyrene-labeled actin assembly was performed at a concentration of 5 μM. Because some polymerization of actin occurs during the freezing of samples, thawed actin was spun at 312,000 × g for 40 min before initiating assembly. Polymerization was initiated at the following final conditions: 5 mM Tris, pH 7.5, 0.5 mM ATP, 2 mM MgCl₂, 10 mM KCl, and 0.2 mM DTT. Fluorescence was monitored continuously: excitation wavelength = 355 nm; emission wavelength = 407 nm. Purified yeast cofolin was used as a control according to Moen et al. (18).

RESULTS

Immunogold Labeling of β-Actin Isoform—Previous studies revealed a polarized distribution of ezrin in the apical and canalicular plasma membranes of gastric parietal cells (6) and that ezrin was spatially co-distributed with actin, specifically within parietal cells along the intracellular canalicular (IC), which are invaginations of the apical surface membrane coursing throughout the parietal cell. Gold particles are also seen along the basolateral region (BI) of parietal cells, with a somewhat lower density than along canalicular. Large mitochondria throughout the cytoplasm are characteristic of parietal cells; numerous cytoplasmic tubulovesicles and canalicular microvilli, also characteristic of parietal cells, are not easily visualized at this magnification. Bar marker is 2 μm.
cells, mucous neck cells, and chief cells) and along the canalicul surface of parietal cells. The secretory canaliculi are invaginations of the apical surface membrane coursing throughout the parietal cell; at the level of magnification shown in Fig. 1, the collapsed canaliculi are easily visualized by the dense trail of enhanced gold particles. Gold particles are also clearly seen along the basolateral region of parietal cells, with a somewhat lower density than along the canaliculi. With the exception of discrete staining along their apical borders, there was virtually no gold staining within chief cells or at their basolateral surfaces. For stimulated gastric glands (Fig. 2), enhanced immunogold staining of β-actin can readily be seen along the dilated canaliculi of parietal cells and the apical surfaces of neighboring epithelial cells. Mitochondria within the stimulated parietal cells appeared to be highly concentrated in the cytoplasm, due to the fusion of tubulovesicles with the apical canalicular membrane. At the higher resolution shown in Fig. 3, specific gold particle labeling of β-actin can clearly be localized to apical microvilli and the region of the terminal web just beneath the apical canalicular surface of parietal cells. Very few gold particles extend deep into parietal cell cytoplasm, although lateral folds and basal membranes show distinct gold labeling.

Gastric Ezrin Is Preferentially Co-sedimented with Cytoplasmic β-Actin—The terminal step in our procedure for purifying ezrin from gastric homogenates is represented in Fig. 4. Two major peaks of ezrin were eluted from the S-Sepharose, one in the range of 450–550 mM NaCl with several ezrin breakdown products in the 40–55 kDa range (identified by blot) and a second peak eluting at 625–675 mM NaCl containing a single 80-kDa ezrin peak and some low molecular weight peptides migrating near the dye front. This second highly purified ezrin preparation was used in our tests of ezrin-actin interaction.

To determine whether gastric ezrin stably binds to actin filaments in an isoform-specific manner, we assayed the ability of ezrin to co-sediment with skeletal muscle α-actin and RBC cytoplasmic β-actin. Gastric ezrin was incubated with the respective actin isoforms under polymerizing conditions for 2 h, and the filaments were pelleted by centrifugation at 312,000 × g for 40 min. Actin filaments longer than 10 subunits in length will sediment under these conditions (19). The Coomassie Blue-
When the molar ratio of actin:ezrin was 20:1, or as actin was increased more ezrin was removed from the supernatant; when the molar ratio of actin:ezrin falls below 10:1, ezrin content in the supernatant increased, consistent with a saturation iso- stochiometry of ezrin-actin association, we carried out separate sets of co-sedimentation experiments: one case in which the ezrin concentration was held constant while varying the actin concentration; in the other case the actin concentration was fixed while ezrin concentration was varied, consistent with a saturation iso-stochiometry of ezrin-actin association.

Gastric Ezrin Affects Pyrene-labeled Actin Polymerization in Vitro—To evaluate the quality of our purified actin isoforms by guest on July 25, 2018http://www.jbc.org/Downloaded from when pyrene-labeled actin subunit is incorporated into a polymerizing filament. Both skeletal $\alpha$-actin and cytoplasmic $\beta$-actin isoforms readily polymerized when KCl and MgCl$_2$ were included as demonstrated by the respective standard curves in Fig. 8, A and B. To validate the properties of our pyrene-labeled actin, we tested the influence of yeast coflin on pyrene-labeled actin (18). Yeast coflin increased the rate of assembly of both skeletal $\alpha$-actin and cytoplasmic $\beta$-actin isoforms. The effects of coflin were most prominent in the late stages of polymerization, after the lag phase (nucleation phase), consistent with the report of Moon et al. (18).

When gastric ezrin was included with the pyrene-labeled skeletal $\alpha$-actin, the curve shifted slightly to the left in the late stages of polymerization, after the lag phase (nucleation phase), consistent with the report of Moon et al. (18). Yeast coflin increased the rate of assembly of both skeletal $\alpha$-actin and cytoplasmic $\beta$-actin isoforms. The effects of coflin were most prominent in the late
achieve steady state decreased from
manner, profile of ezrin on RBC

Ezrin differentially alters the polymerization kinetics of α-actin and β-actin isoforms. Aliquots of pyrene-labeled skeletal α-actin (A) or RBC β-actin (B) were preincubated alone, with purified yeast cofilin, or with several concentrations of purified gastric ezrin as described under “Materials and Methods.” Assembly was initiated at zero time by polymerization medium to a final concentration of 2 mM MgCl2, 10 mM KCl, and 0.5 mM ATP. Assembly was followed by changes in fluorescence of the pyrene-labeled actins over time. Excitation = 355 nm; emission = 407 nm. In all cases the final concentration of actin was 5 μM. Assembly of either α-actin or β-actin alone is shown by solid lines. Incubation with cofilin at 1:16 molar ratio (cofilin/actin) is shown by dotted lines. Incubations with various ezrin/actin molar ratios (as indicated) are shown by the dashed lines.

stage of polymerization, but not in the initial nucleation stage, suggesting that ezrin might bind to the actin filaments or possibly promote the assembly (Fig. 8A). An increased molar ratio of ezrin/actin further shifted the curve to the left.

The addition of ezrin also promoted the assembly of pyrene-labeled β-actin, with effects that were more pronounced than for skeletal α-actin (Fig. 8B). Ezrin reduced the time for assembly to achieve steady state in a concentration-dependent manner, e.g. at a molar ratio of 1:10 (ezrin:actin) the time to achieve steady state decreased from ~60 to ~30 min. The profile of ezrin on RBC β-actin assembly is somewhat like that of cofilin, that is, ezrin does not seem to modulate the lag associated with the nucleation phase while it promotes the filament elongation phase as evidenced by increased rate of assembly after the initial lag phase. Thus the pyrene-labeled actin assembly assay indicated that ezrin might directly interact with actin isoforms, with a distinct preference for RBC β-actin compared with skeletal α-actin.

DISCUSSION

Two different actin isoforms have been identified within parietal cells, cytoplasmic β-actin and cytoplasmic γ-actin, which are polarized to the apical and basolateral membranes, respectively (4). In addition these studies demonstrated a preferential interaction between ezrin and the β-actin isoform extracted from native parietal cells. In the present studies, we extended our earlier finding by using immunoelectron microscopy, we localized β-actin to the canalicular surface and apical microvilli where gastric ezrin is enriched (6). Despite the dramatic elongation of apical microvilli and dilation of the canalicular lumina of parietal cells during acid secretion, the immunogold labeling of β-actin did not reveal obvious stimulation-mediated redistribution of β-actin. In fact, a recent report on the state of actin in resting and stimulated gastric glands using the DNase I assay did not reveal any significant change in either filamentous or monomeric actin pool (21), suggesting that stimulation-mediated elongation of microvilli could be due mainly to an invagination of plasma membrane resulting from the fusion of tubulovesicular membrane. It is possible that the stability and integrity of microfilaments are required for providing a structural support for the dynamic extension of apical plasma membrane and growth of microvilli.

The three-dimensional structure of cytoplasmic β-actin was recently solved in complex with profilin (22). Although its primary sequence is generally similar to skeletal α-actin, cytoplasmic β-actin displays several structural differences. These include: the N-terminal conformation of β-actin bearing a turn rather than the helical structure in skeletal α-actin, distinct rotational differences within subdomains of the isoforms, and differences in side chain orientation at residues 38–52. Physiological interpretation of these structural differences is still under debate (23), but functional distinctions for the interaction of actin isoforms with actin binding proteins have been reported. Larsson and Lindberg (2) showed that cytoplasmic β-actin and γ-actin have higher affinity to bind profilin (Kd ~10^-8 M) than that of sarcomeric actin (Kd ~4 x 10^-6 M) and that profilin interaction with the non-muscle isoforms was regulated by Mg2+. Rozyczki et al. (3) further demonstrated that profilin preferentially binds to cytoplasmic β-actin compared with γ-actin. The increased ratio of β- to γ-actin isoforms during the course of co-purifying ezrin and actin by immunoprecipitation hinted that β-actin filaments might preferentially bind to ezrin (4). Bretschler observed (11) that ezrin co-sedimented with filamentous skeletal muscle α-actin in vitro only at low ionic strength. Our studies with the comparison of two different actin isoforms clearly show that gastric ezrin preferentially binds to cytoplasmic β-actin while ezrin binds poorly to the skeletal α-actin.

A recent series of studies has provided further information concerning the structural and functional relevance of closely related ERM protein family members. Martin et al. (24) showed that the N-terminal domain of ezrin inhibits the functional activity of cell surface protrusion exerted by the C-terminal domain. These authors further mapped the inhibitory domain to the first 115 N-terminal residues. A similar interaction between the N- and C-terminal domains was also observed by Henry et al. (25) in their study of the functional relevance of radixin. Gary and Bretschler (26, 27) carried out a meticulous characterization of homotypic and heterotypic interaction among the ERM protein family members, specifically ezrin and moesin. They established conditions under which there is heterotypic interaction between the N-terminal domain (amino acids 1-296) and C-terminal domain (amino acids 479–585) to form dimers and possibly oligomers. These terminal “interactive domains” were clearly demonstrated in an extensive set of test probings using fusion protein constructs of the respective N- and C-terminal interactive domains. However, for the full-length fusion protein, or for isolated native ezrin, they interpreted their results to suggest that the monomer exists in a
form in which the C-terminal interactive domain is masked by a folding that also partially obscures the N-terminal interactive domain. When full-length ezrin was denatured the C-terminal interactive domain became accessible, but the N-terminal domain was inactivated by denaturation. Because the actin binding domain of ezrin is near the C terminus (28), and the apparent masking of the C-terminal interactive domain in the full-length monomer, Gary and Bretscher offered these data as a basis to explain the lack of ezrin association with α-actin filaments in vitro (11). It is possible that modification (e.g., phosphorylation) and accessory proteins might perturb this masking process and expose the F-actin binding site in the C-terminal domain. In fact, purified ezrin from gastric mucosa contains multiple spots as resolved by two-dimensional electrophoresis, which suggests that native gastric ezrin might contain a pool of phospho ezrin and/or multiple isoforms. Since there are three conserved phosphorylation sites for protein kinase A in ezrin (Ser^65, Thr^213, and Thr^323), and protein kinase A-mediated phosphorylation has been implicated in hormone-stimulated acid secretion (8), it is conceivable that the phosphorylation might alter the intramolecular masking effect and expose the C-terminal domain for actin binding. In fact, phosphorylation of ezrin by epidermal growth factor receptor tyrosine kinase triggered the dimerization, although the functional activity of this dimer is unknown at present (27). Alternatively, ezrin isoforms might form functional oligomers that might exert F-actin-binding action.

Shuster and Herman (10) recently reported that ezrin preferentially binds to an affinity column made of filamentous erythrocytic β-actin, but not to a skeletal α-actin. Because these authors were unable to reconstitute erythrocytic β-actin-ezrin interaction in vitro, they concluded that their observed interaction was indirect, and suggested that a 73-kDa polypeptide served to link the interaction between ezrin and filaments of β-actin isoform, although other candidates were also possible. Data presented here would argue against a requirement for the 73-kDa component, since there was no such peptide present in our reaction mixtures. On the other hand, it is not possible to rule out the participation of a low molecular weight component in the ezrin-β-actin filament interaction.

There are some apparent contradictions concerning the nature of ezrin-actin interaction. Based on the displacement of ezrin from actin filaments by cytochalasin D, Shuster and Herman (10) proposed that ezrin binds to the barbed ends of actin filaments. Using a blot overlay assay, Pestonjamsap et al. (29) found that immobilized ezrin bound to actin filaments and the binding was minimized by myosin S1 subfragment but not by gelsolin or capping protein, suggesting that binding occurs at the filament sides and not at barbed ends. Moreover, the parallel localizations of ezrin and F-actin at the light and EM levels (6, 7, 11) are consistent with side binding. Our studies of ezrin on pyrene-labeled actin assemble in vitro also favors the idea that ezrin interacts with actin filaments along the side since ezrin dose-dependently promotes actin assembly, which is typically seen for a side-binding polypeptide, myosin S1 fragment (30, 17). Recently, Turunen et al. (28) revealed that the region 558–578 in ezrin, moesin, and radixin shows high sequence homology with the actin binding site of CapZ β-subunit (31). Despite the fact that both radixin and CapZ β-subunit bind to the barbed ends of actin filaments (31, 32), it is not clear whether the homologous region which exerts actin binding for ezrin is also responsible for the end-binding in the case of CapZ and radixin. Recent studies showed that EF1α, a transcription factor, has typical barbed end capping activity in addition to the known actin filament side-binding property (33). Thus, we cannot rule out the possibility that ezrin might interact with the ends of actin filaments at present.

In summary, our immunoelectron microscopic data show cytoplasmic β-actin is indeed located to the apical microvilli and terminal web of parietal cells, in a pattern identical to what has been reported for ezrin (4, 6, 7). We have verified that gastric ezrin is preferentially associated with cytoplasmic β-actin filaments in vitro, compared with skeletal α-actin, and that gastric ezrin binds to β-actin filaments in a saturable manner. Finally, based on the pyrene-labeled actin assembly data, we speculate that ezrin might modulate the elongation phase of the actin assembly. Because of the isoprefential associations demonstrated in this study and morphological separations seen in several systems (34–36), we suggest that actin isoforms might segregate into different functional domains and exert their specificities by interacting with isoform-oriented actin-binding proteins.

Acknowledgments—We thank Dr. Christine Chapponnier for providing antibody against cytoplasmic β-actin, Dr. Ann Moon for purified yeast cellin, and Dr. Roger Cooke for S1 subfragment. We extend special thanks to Dr. Kent McDonald for his valuable assistance with electron microscopy and for his critical reading of the manuscript.

REFERENCES

1. Vandekerckhove, J., and Weber, K. (1978) J. Mol. Biol. 126, 783–802.
2. Larsson, H., and Lindberg, U. (1988) Biochim. Biophys. Acta 953, 95–105.
3. Rozycki, M., Schutt, C. E., and Lindberg, U. (1991) Methods Enzymol. 196, 109–118.
4. Yao, X., Chapponnier, C., Gabbiani, G., and Forte, J. G. (1995) Mol. Biol. Cell 6, 541–557.
5. Yoneyama, S., Nagafuchi, A., Sato, N., and Tsukita, S. (1990) J. Cell Biol. 120, 437–449.
6. Hanzel, D., Reggio, H., Bretscher, A., Forte, J. G., and Mangeat, P. (1991) EMBO J. 10, 2363–2373.
7. Pestonjamsap, K., Hanzel, D., and Forte, J. G. (1990) Am. J. Physiol. 256, G1082–G1089.
8. Cooper, J. A., Walker, S. B., and Pollard, T. D. (1983) J. Cell Biol. 97, 425–432.
9. Pardee, J. D., and Spudich, J. A. (1982) J. Mol. Biol. 24, 271–289.
10. Rozycki, M., Schutt, C. E., and Lindberg, U. (1990) Biochim. Biophys. Acta 105, 205–220.
11. Bretscher, A. (1986) Methods Enzymol. 154, 24–37.
12. Yao, X., Thibodeau, A., and Forte, J. G. (1993) J. Am. Physiol. 265, C36–C46.
13. Berryman, M., Franck, Z., and Bretscher, A. (1993) J. Cell Biol. 105, 1025–1043.
14. Kouyama, T., and Mihashi, K. (1981) Eur. J. Biochem. 114, 33–38.
15. Yao, X., Chaponnier, C., Gabbiani, G., and Forte, J. G. (1995) Eur. J. Biochem. 2363–2373.
16. Molea, L., and Janney, P. A., Louie, K. A., and Drubin, D. G. (1993) J. Cell Biol. 120, 421–435.
17. Attri, A. K., Lewis, M. S., and Korn, E. D. (1991) J. Biol. Chem. 266, 6815–6824.
18. Cooper, J. A., Walker, S. B., and Pollard, T. D. (1983) J. Muscle Res. Cell Motil. 4, 253–262.
19. Oghara, S., Ramilo, M., Yao, X., Ly, B., and Forte, J. G. (1995) Mol. Biol. Cell 6, 371a.
20. Pestonjamsap, K., Amieva, M. R., Strassel, C. P., Nauseef, W. M., Furthmayr, H., and Luna, E. J. (1995) Mol. Biol. Cell 6, 247–259.
21. Kow, Y., Mihashi, K., and Hanzel, D. (1990) Eur. J. Biochem. 201, 87–95.
22. Cooper, J. A., Walker, S. B., and Pollard, T. D. (1983) J. Muscle Res. Cell Motil. 4, 253–262.
23. Martin, M., Andreoli, C., Salahu, P. L., and Santolou, A. (1989) Nature 340, 801–816.
24. Rozycki, M. D., Mylly, J., Schutt, C. E., and Lindberg, U. (1994) Curr. Opin. Cell Biol. 6, 87–95.
25. Faivre, J., Mangeat, P., and Mangeat, P. (1995) J. Cell Biol. 128, 1081–1093.
26. Henry, M. D., Agosti, C. G., and Solomon, F. J. (1995) J. Cell Biol. 129, 1007–22.
27. Gary, R., and Bretscher, A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10846–10850.
28. Gary, R., and Bretscher, A. (1995) Mol. Biol. Cell 6, 1061–1075.
29. Turunen, O., Wahlström, T., and Vaheri, A. (1994) J. Cell Biol. 126, 1445–1453.
30. Pestonjamsap, K., Amieva, M. R., Strassel, C. P., Nauseef, W. M., Furthmayr, H., and Luna, E. J. (1995) Mol. Biol. Cell 6, 247–259.
31. Kow, Y., Mihashi, K., and Hanzel, D. (1990) Eur. J. Biochem. 201, 87–95.
32. Cooper, J. A., Walker, S. B., and Pollard, T. D. (1983) J. Muscle Res. Cell Motil. 4, 253–262.
