Phosphoinositide Binding Regulates α-Actinin Dynamics
MECHANISM FOR MODULATING CYTOSKELETAL REMODELING*

Received for publication, January 18, 2005, and in revised form, February 10, 2005
Published, JBC Papers in Press, February 13, 2005, DOI 10.1074/jbc.M500631200

Tamara S. Fraley‡, Clifford B. Pereira‡, Thuan C. Tran‡, CoreyAyne Singleton‡, and Jeffrey A. Greenwood‡¶

From the ‡Department of Biochemistry and Biophysics and the ¶Department of Statistics, Oregon State University, Corvallis, Oregon 97331

The active association-dissociation of dynamic protein-protein interactions is critical for the ability of the actin cytoskeleton to remodel. To determine the influence of phosphoinositide binding on the dynamic interaction of α-actinin with actin filaments and integrin adhesion receptors, fluorescence recovery after photobleaching (FRAP) microscopy was carried out comparing wild-type green fluorescent protein (GFP)-α-actinin and a GFP-α-actinin mutant with a decreased affinity for phosphoinositides (Fraley, T. S., Tran, T. C., Corgan, A. M., Nash, C. A., Hao, J., Critchley, D. R., and Greenwood, J. A. (2003) J. Biol. Chem. 278, 24039–24045). In fibroblasts, recovery of the mutant α-actinin protein was 2.2 times slower than the wild type along actin stress fibers and 1.5 times slower within focal adhesions. FRAP was also measured in U87MG glioblastoma cells, which have higher levels of 3-phosphorylated phosphoinositides. As expected, α-actinin turnover for both the stress fiber and focal adhesion populations was faster in U87MG cells compared with fibroblasts with recovery of the mutant protein slower than the wild type along actin stress fibers. To understand the influence of α-actinin turnover on the modulation of the actin cytoskeleton, wild-type or mutant α-actinin was co-expressed with constitutively active phosphoinositide (PD) 3-kinase. Co-expression with the α-actinin mutant inhibited actin reorganization with the appearance of enlarged α-actinin containing focal adhesions. These results demonstrate that the binding of phosphoinositides regulates the association-dissociation rate of α-actinin with actin filaments and integrin adhesion receptors and that the dynamics of α-actinin is important for PI 3-kinase-induced reorganization of the actin cytoskeleton. In conclusion, phosphoinositide regulation of α-actinin dynamics modulates the plasticity of the actin cytoskeleton influencing remodeling.

The dynamic nature of the actin cytoskeleton is essential for various aspects of intracellular physiology and function as well as the ability of the cell to respond to signals stimulating cellular processes such as proliferation, differentiation, and migration. Individual protein molecules exchange between structures and the cytoplasm and/or membrane keeping the actin cytoskeleton pliable and able to quickly respond to signals inducing reorganization. In this report, we will refer to this dynamic exchange of proteins as turnover. Much of what we know about actin cytoskeletal dynamics has focused on the regulation of actin polymerization by specific actin-binding proteins during lamellipodial formation at the leading edge of migrating cells (1). It is largely unknown how actin filament bundling proteins influence actin cytoskeletal dynamics and remodeling in the cell. These proteins have not been thought to play an active role in the modulation of actin organization but have rather been cast in the more passive part as stabilizers of an actin cytoskeleton organized by the proteins regulating actin polymerization. In this study, we focus on the influence of α-actinin, an actin filament bundling protein, on the remodeling of the actin cytoskeleton.

As an actin bundling protein that provides a direct link between actin stress fibers and integrin receptors within focal adhesions, α-actinin is a key protein for maintaining the stability of the actin cytoskeleton (2, 3). The localization of α-actinin along actin stress fibers occurs through direct interaction of the actin binding domains of the anti-parallel homodimer with actin microfilaments (2, 4–6). α-Actinin is localized to focal adhesions primarily as a result of direct interactions with actin filaments within the terminating stress fiber and integrin adhesion receptors (2, 7–9). Fluorescence recovery after photobleaching (FRAP) microscopy of α-actinin along stress fibers measures the association-dissociation rate of α-actinin with actin filaments within living cells. Similarly, FRAP of α-actinin within focal adhesions is a combined measurement of the association-dissociation rate of α-actinin with actin filaments and integrins.

Although the dynamics of α-actinin in cultured cells has recently been studied by several groups imaging GFP-α-actinin using FRAP (10–12), it is not known what factors regulate the rate of α-actinin turnover. Many actin-binding proteins are regulated by specific interactions with phosphoinositides modulating activity and localization (13), and we have demonstrated that α-actinin is regulated by phosphoinositide binding (14–16). More specifically, we have shown that the binding of PtdIns(4,5)P2 and PtdIns(3,4,5)P3 inhibit the bundling activity of α-actinin and that PtdIns(3,4,5)P3 binding disrupts α-actinin bundling activity (15, 16) and interaction with the cytoplasmic domain of integrin adhesion receptors (14). Furthermore, replacement of three positively charged amino acid residues

* This work was supported by National Institutes of Health Grant GM 63711 (to J. A. G.) from the NIGMS. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† To whom correspondence should be addressed: Dept. of Biochemistry and Biophysics, ALS 2011, Oregon State University, Corvallis, OR 97331. Tel.: 541-737-4997; Fax: 541-737-0481; E-mail: jeffrey.greenwood@orst.edu.

1 The abbreviations used are: FRAP, fluorescence recovery after photobleaching; PtdIns, phosphatidylinositol; GFP, green fluorescent protein; REF, rat embryonic fibroblast; τ1/2, half-time of recovery; PI 3-kinase, phosphoinositide 3-kinase; PBS, phosphate-buffered saline; PDGF, platelet-derived growth factor.
within the phosphoinositide bonding site of α-actinin decreased binding to phosphoinositides by as much as 80% (15). Cells overexpressing the mutant protein were observed to have an increase in bundled actin filaments altering the organization of the actin cytoskeleton (15). Although these results demonstrated that phosphoinositide regulation of α-actinin bundling activity was modulating the structure of the actin cytoskeleton, the mechanism by which phosphoinositides were regulating α-actinin in the cell was not clear. In this study, we used the GFP-α-actinin wild-type and mutant proteins to examine the regulation of α-actinin turnover by phosphoinositide binding. The results show that phosphoinositide binding regulates the association-dissociation rate of α-actinin with actin filaments and integrin adhesion receptors influencing the plasticity of the actin cytoskeleton and its ability to remodel.

MATERIALS AND METHODS

FRAP Microscopy—FRAP experiments were carried out consistent with previously described procedures (11, 12, 17). Rat embryonic fibroblasts (REFs) and U87MG glioblastoma cells were cultured in 10% fetal bovine serum-Dulbecco’s modified Eagle’s medium on 35-mm glass-bottom dishes (MatTek Corporation) and transfected with pEGFP-α-actinin wild-type and mutant constructs as described previously (15). One day after transfection, cells were transferred to the heated stage (37°C ± 0.1°C, flow of a Zeiss LSM510 confocal microscope and equilibrated for at least 1 h. GFP fluorescence was imaged with a ×63 oil immersion objective with objective heater using the 488-nm line of a 30-milliwatt argon laser, and emissions were collected using a LP505 filter. Images were acquired at full dynamic range using the lowest laser intensity possible allowing the collection of quality images with little or no intrinsic photobleaching during the recovery period. Stationary cells expressing GFP-α-actinin of equivalent intensity and morphology were selected for the FRAP experiments. To prevent damage to the cells from the laser, only one region of interest (average size was 45 μm² for stress fibers and 13 μm² for focal adhesions) was chosen per cell. Five images were taken prior to photobleaching to calculate the initial fluorescence intensity. One hundred and fifty iterations with the laser were needed to photobleach the region of interest to ~10% of the initial fluorescence. Following photobleaching, images were collected every 15 s for 15 min for the REFs and 10 min for the U87MGs. Photobleaching of the stress fibers had no observable negative effects on the cytoskeleton or morphology of the cell. However, photobleaching of peripheral focal adhesions did, on occasion, cause some retraction of the cell. This effect was observed for cells, primarily the U87MGs, expressing both wild-type and mutant GFP-α-actinin, and these experiments could not be completed. Fluorescence intensity was quantified using Zeiss Physiological Software 3.2.

For each recovery curve, the half-time of recovery ($t_{1/2}$) was the time taken to reach 50% of the postbleach maximum. To remove the effect of random fluctuations in fluorescence intensity, each postbleach maximum was estimated from the nonparametrically smoothed recovery curve (SAS Loess procedure with smoothing parameter chosen as the global minimum for the AICC criterion after graphical verification). Within the REF and U87MG data sets, the estimated $t_{1/2}$ values were compared between cell types using linear mixed models (SAS mixed procedure) after log transformation of the data to reduce heterogeneity of variance. For REF data, experimental days were included in the model as additive complete blocks (interaction, $p > 0.3$). The final model for REF data allowed mutant cells to have different (greater) variability than wild-type cells ($p < 0.005$, residual likelihood ratio test) leading to the use of the Kenward-Roger's adjustment and Satterthwaite approximation for standard errors and tests. For U87MG data the final model was a simple one-way analysis of variance because data were collected on a single day and the variances were reasonably homogeneous after log transformation (Levene’s test, $p > 0.12$). Residuals were acceptable for both sets of data. All statistical analyses were done with SAS version 9.1.2. The mobile fraction was calculated as described by Tsuruta et al. (11).

Fluorescence Microscopy—REFs were co-transfected with pEGFP-α-actinin wild type or mutant and p110K227E 5'-Myc or, as a control, the empty pSG5 construct (19). One day after transfection, cells were fixed with 3% formaldehyde (Tousimis) in PBS for 30 min at room temperature. After fixation, cells were washed with PBS, permeabilized with 0.5% Triton X-100 in PBS, blocked with 1% BSA in PBS, and stained with anti-Myc (Upstate) followed by rhodamine-conjugated secondary antibody (Jackson Laboratories) to identify PI 3-kinase-expressing cells and Alexa 350-phalloidin (Molecular Probes) to visualize filamentous actin. Images of the cells were acquired using a Zeiss Axiosvert 100S microscope with a Photometrics CoolSNAP HQ CCD camera.

RESULTS AND DISCUSSION

To determine the influence of phosphoinositide binding on the association-dissociation rate of α-actinin with actin filaments, FRAP microscopy was carried out comparing wild-type GFP-α-actinin and the GFP-α-actinin mutant with a decreased affinity for phosphoinositides (15). Both proteins were transiently expressed in REFs as previously characterized (15). A region of interest containing α-actinin bundled stress fibers within stationary cells was photobleached, and fluorescence recovery quantified within the defined region (Fig. 1). We observed a homogeneous recovery of GFP-α-actinin along the stress fibers with a $t_{1/2}$ of 51.1 s and ~90% of the fluorescence recovered by 300 s (Figs. 1 and 2, Table I). These rates are within the range of those previously reported for GFP-α-actinin using different cellular systems and culturing conditions (10–12, 20). Recovery of the GFP-α-actinin mutant was 2.2 times slower than the wild-type protein with a $t_{1/2}$ of 111.9 s and less than 80% of the fluorescence recovered by 300 s (Figs. 1 and 2, Table I). Experiments were carried out on four separate days comparing cells expressing the wild-type or mutant protein; the $t_{1/2}$ was consistently slower for the GFP-α-actinin mutant compared with the wild-type protein ($p < 0.0001$(Fig. 2B). The mobile fractions for wild-type and mutant α-actinin were comparable (Table I), indicating that phosphoinositide binding regulates the rate of turnover and not the ability of the protein to exchange.

These results suggest that the binding of phosphoinositides regulates the association-dissociation rate of α-actinin with the actin filaments within stress fibers. An important question is whether the population of α-actinin bundling the actin filaments in stress fibers is accessible to and can interact with membrane-localized phosphoinositides. In addition to the above results and our previous study examining the GFP-α-actinin mutant (15), Woods and colleagues (21) recently demonstrated that α-actinin interacted with the transmembrane receptor syndecan 4 along actin stress fibers. Furthermore, using immunofluorescent stain-
Phosphoinositide binding regulates the association-dissociation rate of α-actinin with actin filaments and integrins within focal adhesions. REFs expressing GFP-α-actinin wild type or mutant with equivalent fluorescence intensity and similar morphologies were chosen for the FRAP experiments. One region of interest containing a focal adhesion was selected for each cell. FRAP experiments were carried out as described under “Materials and Methods.” An image of the cell prior to photobleaching is shown with the region of interest. Images of the region of interest immediately following photobleaching and after 30 and 300 s of recovery are also shown. Bar = 10 μm.

To further examine the role of phosphoinositides in regulating α-actinin dynamics, turnover was measured in U87MG glioblastoma cells, which do not express the lipid phosphatase PTEN (26) and thus have higher levels of the 3-phosphorylated phosphoinositides compared with cells expressing the phosphatase (27). As expected, α-actinin turnover for both the stress fiber and focal adhesion populations was faster in U87MG cells compared with REFs with recovery of the wild-type protein faster than the mutant along actin stress fibers (Table I). These results further support the hypothesis that phosphoinositide binding regulates the turnover of α-actinin in live cells. Interestingly, the wild-type and mutant proteins recovered at the same rate within the focal adhesions of U87MG cells. It is not clear why a rate difference was observed between the wild-type and mutant proteins in the focal adhesions of REFs but not U87MGs.

To determine the influence of phosphoinositide-regulated α-actinin turnover on the remodeling of the actin cytoskeleton, REFs were co-transfected to express wild-type or mutant GFP-α-actinin with constitutively active PI 3-kinase. Rodriguez-Viciana et al. (28) demonstrated that expression of constitutively active PI 3-kinase in fibroblasts resulted in increased actin dynamics, Fukami et al. (22) reported co-localization of PtdIns(4,5)P₂ and α-actinin along actin stress fibers. These results suggest that this population of α-actinin is in close enough proximity to bind membrane phosphoinositides.

We next examined the association-dissociation rate of α-actinin with actin filaments and integrin receptors within focal adhesions. FRAP assays of GFP-α-actinin within focal adhesions were carried out exactly as described for the stress fiber experiments on the same days using different cells in the same culture dishes (Fig. 3). The size and distribution of the focal adhesions in the REFs expressing both the wild-type and mutant proteins were heterogeneous from cell to cell. As previously reported (15), structural differences have not been identified in the focal adhesions of unstimulated REFs expressing wild-type α-actinin compared with the mutant protein. Recovery of α-actinin within focal adhesions was 1.3 times faster than stress fibers (p = 0.0062, Table I). A similar trend was observed by Edlund et al. (12), although reported as statistically insignificant. The turnover of α-actinin within focal adhesions was 1.5 times slower for the mutant protein (t_{1/2} = 57.6 s) compared with the wild-type (t_{1/2} = 39.7 s) (p = 0.04). These results suggest that phosphoinositide binding regulates the association-dissociation rate of α-actinin with actin filaments and integrin receptors within focal adhesions, although a greater influence was observed on the association-dissociation rate of α-actinin with actin filaments within stress fibers. It is not clear why the rate of turnover for the α-actinin population within focal adhesions is faster compared with stress fibers; however, several factors may contribute. The increased rate of turnover may result from the local production of phosphoinositides by phosphatidylinositol phosphate kinase and PI 3-kinase, which have been shown to localize to focal adhesions (23, 24). The focal adhesion population of α-actinin would also be expected to be in closer proximity to the membrane increasing the potential for interaction with phosphoinositides. In addition, the affinity of α-actinin is higher for actin filaments than integrin receptors (25).

Figure 2. Analysis of FRAP experiments examining the turnover of α-actinin along actin stress fibers. A, recovery curves for GFP-α-actinin wild type (top trace) and mutant (bottom trace), n = 12–13; error bars represent S.E. B, the t_{1/2} of wild-type (●) and mutant (○) GFP-α-actinin is plotted for each cell by experimental day; the GFP-α-actinin mutant consistently recovered at a slower rate compared with the wild type. Two of the data points for the wild-type cells on day 3 are overlapping.

**Table I**

|          | n  | t_{1/2} (s) | Mobile fraction | p       | S.E. |
|----------|----|-------------|-----------------|---------|------|
| REFs     |    |             |                 |         |      |
| Wild-type-SF | 12 | 51.1 ± 3.67 | 88.1 ± 5.63     | >0.0001 |      |
| Mutant-SF | 12 | 111.9 ± 9.36| 91.9 ± 5.02     | <0.0001 |      |
| Wild-type-FA | 12 | 39.7 ± 2.99 | 90.1 ± 2.83     |         |      |
| Mutant-FA | 10 | 57.6 ± 8.41 | 91.9 ± 7.43     | 0.04    |      |
| U87MGs   |    |             |                 |         |      |
| Wild-type-SF | 5  | 31.2 ± 2.27 | 88.6 ± 4.41     |         |      |
| Mutant-SF | 6  | 55.9 ± 5.40 | 94.7 ± 6.36     | <0.0001 |      |
| Wild-type-FA | 8  | 27.1 ± 0.72 | 87.3 ± 4.71     |         |      |
| Mutant-FA | 6  | 29.0 ± 1.31 | 86.5 ± 6.06     | 0.04    |      |

**FIG. 1.** FRAP analysis

Data are presented with standard error of the mean (S.E.) n, number of cells evaluated. t_{1/2}, half-time of recovery. Statistical comparisons (p column) were made between the wild-type and mutant stress fibers (SF) or focal adhesions (FA) from REFs and U87MG glioblastoma cells.

**FIG. 2.** Analysis of FRAP experiments examining the turnover of α-actinin along actin stress fibers. A, recovery curves for GFP-α-actinin wild type (top trace) and mutant (bottom trace), n = 12–13; error bars represent S.E. B, the t_{1/2} of wild-type (●) and mutant (○) GFP-α-actinin is plotted for each cell by experimental day; the GFP-α-actinin mutant consistently recovered at a slower rate compared with the wild type. Two of the data points for the wild-type cells on day 3 are overlapping.

**FIG. 3.** Phosphoinositide binding regulates the association-dissociation rate of α-actinin with actin filaments and integrins within focal adhesions. REFs expressing GFP-α-actinin wild type or mutant with equivalent fluorescence intensity and similar morphologies were chosen for the FRAP experiments. One region of interest containing a focal adhesion was selected for each cell. FRAP experiments were carried out as described under “Materials and Methods.” An image of the cell prior to photobleaching is shown with the region of interest. Images of the region of interest immediately following photobleaching and after 30 and 300 s of recovery are also shown. Bar = 10 μm.

To further examine the role of phosphoinositides in regulating α-actinin dynamics, turnover was measured in U87MG glioblastoma cells, which do not express the lipid phosphatase PTEN (26) and thus have higher levels of the 3-phosphorylated phosphoinositides compared with cells expressing the phosphatase (27). As expected, α-actinin turnover for both the stress fiber and focal adhesion populations was faster in U87MG cells compared with REFs with recovery of the wild-type protein faster than the mutant along actin stress fibers (Table I). These results further support the hypothesis that phosphoinositide binding regulates the turnover of α-actinin in live cells. Interestingly, the wild-type and mutant proteins recovered at the same rate within the focal adhesions of U87MG cells. It is not clear why a rate difference was observed between the wild-type and mutant proteins in the focal adhesions of REFs but not U87MGs.

To determine the influence of phosphoinositide-regulated α-actinin turnover on the remodeling of the actin cytoskeleton, REFs were co-transfected to express wild-type or mutant GFP-α-actinin with constitutively active PI 3-kinase. Rodriguez-Viciana et al. (28) demonstrated that expression of constitutively active PI 3-kinase in fibroblasts resulted in increased actin dynamics, Fukami et al. (22) reported co-localization of PtdIns(4,5)P₂ and α-actinin along actin stress fibers. These results suggest that this population of α-actinin is in close enough proximity to bind membrane phosphoinositides.

We next examined the association-dissociation rate of α-actinin with actin filaments and integrin receptors within focal adhesions. FRAP assays of GFP-α-actinin within focal adhesions were carried out exactly as described for the stress fiber experiments on the same days using different cells in the same culture dishes (Fig. 3). The size and distribution of the focal adhesions in the REFs expressing both the wild-type and mutant proteins were heterogeneous from cell to cell. As previously reported (15), structural differences have not been identified in the focal adhesions of unstimulated REFs expressing wild-type α-actinin compared with the mutant protein. Recovery of α-actinin within focal adhesions was 1.3 times faster than stress fibers (p = 0.0062, Table I). A similar trend was observed by Edlund et al. (12), although reported as statistically insignificant. The turnover of α-actinin within focal adhe-
plasticity of the actin cytoskeleton. PtdIns(3,4,5)P$_3$, which is generally produced transiently in response to the activation of specific signaling pathways, has the more active and localized role of disrupting the α-actinin link between the integrin adhesion receptors and actin filaments assisting in the restructuring of focal adhesions and loss of stress fibers.

In summary, we have used an α-actinin mutant with a decreased affinity for phosphoinositides to demonstrate that the binding of phosphoinositides regulates the association-dissociation rate of α-actinin with actin filaments and integrin adhesion receptors in cells. Furthermore, phosphoinositide-regulated α-actinin turnover influenced the plasticity and remodeling of the actin cytoskeleton. As far as we know, this is the first study to show that phosphoinositide binding can regulate protein-protein interaction dynamics in the cell and identifies the regulation of protein turnover as a novel mechanism for modulating the remodeling of the actin cytoskeleton.

Acknowledgments—We thank Dr. P. Andrew Karplus (Oregon State University) for critical reading of the manuscript and Dr. Julian Downward (London Research Institute) for the p110R22T7E 5'-Myc construct. This publication was made possible in part by the Confocal Microscopy (National Institutes of Health Grant 1518–1522–01) and Cell Culture and Statistics Core Facilities of the Environmental Health Sciences Center, Oregon State University, from Grant P30 ES00210, National Institute of Environmental Health Sciences, National Institutes of Health. Special thanks to John N. Giovanni for assistance with the statistical analysis.

REFERENCES

1. Small, J. V., Stradal, T., Vignal, E., and Rottner, K. (2002) Trends Cell Biol. 12, 112–120
2. Otey, C. A., and Carpen, O. (2004) Cell Mol. Clocksytok. 58, 104–111
3. Rajfer, Z., Roy, P., Otey, C., Romer, L., and Jacobson, K. (2002) Nat. Cell Biol. 4, 298–303
4. Hemmings, L., Kuhlman, P. A., and Critchley, D. R. (1992) J. Cell Biol. 116, 1389–1390
5. Blanchard, A., Anhian, V., and Critchley, D. R. (1989) J. Muscle Res. Cell Motil. 10, 280–289
6. Bhatti, A., Kaverina, I., Otey, C., and Huttonlocher, A. (2002) J. Cell Sci. 115, 3415–3425
7. Pavalko, F. M., Otey, C. A., Simon, K. O., and Burridge, K. (1991) Biochem. Soc. Trans. 19, 1005–1009
8. Lyman, S., Gilmore, A., Burridge, K., Gidwitz, S., and White, G. C., II (1997) J. Biol. Chem. 272, 22538–22545
9. Lewis, J. M., and Schwartz, M. A. (1995) Mol. Biol. Cell 6, 151–160
10. Peterson, L. J., Rajfer, Z., Maddox, A. S., Freel, C. D., Chen, Y., Edlund, M., and Burridge, K. (2004) J. Biol. Cell. 15, 3497–3508
11. Tsubura, D., Gonzales, M., Hopkinson, S. B., Otey, C., Khun, S., Goldman, R. D., and Jones, J. C. (2002) FASEB J. 16, 866–868
12. Edlund, M., Lotano, M. A., and Otey, C. A. (2001) Cell Motil. Cytoskeleton 48, 190–200
13. Yin, H. L., and Janmey, P. A. (2003) Annu. Rev. Cell Biol. 65, 761–789
14. Greenwood, J. A., Theibert, A. B., Prestwich, G. D., and Murphy-Ullrich, J. E. (2000) J. Cell Biol. 150, 627–642
15. Fraley, T. S., Tran, T. C., Corgan, A. M., Nash, C. A., Hao, J., Critchley, D. B., and Greenwood, J. A. (2003) J. Biol. Chem. 278, 24039–24045
16. Corgan, A. M., Singleton, C., Santoso, C. B., and Greenwood, J. A. (2004) Biochem. J. 376, 1067–1072
17. van Drogen, F., and Peters, M. (2004) Methods Mol. Biol. 284, 287–306
18. Shimizu, Y., and Hunt, S. W., III (1996) EMBO J. 15, 287–306
19. Rodriguez-Viciana, P., Warne, P. H., Vanhaesebroeck, B., Waterfield, M. D., and Downward, J. (1999) EMBO J. 18, 2442–2451
20. von Wichert, G., Haimovich, B., Feng, G. S., and Sheetz, M. P. (2003) EMBO J. 22, 5023–5035
21. Greene, B. K., Tumova, S., Couchman, J. R., and Woods, A. (2003) J. Biol. Chem. 278, 7617–7623
22. Fuchiki, K., Endo, T., Imamura, M., and Takenawa, T. (1994) J. Biol. Chem. 269, 1518–1522
23. Gillham, H., Goldberg, M. C., Pepperkok, R., and Gullick, W. J. (1999) J. Cell Biol. 146, 869–880
24. Lang, K., Doughton, R. L., Firestone, A. J., Bunce, M. W., and Anderson, R. A. (2002) PLoS Medicine 22538–22547
25. Mills, J. A., and Firestone, A. J. (2003) Methods Mol. Biol. 284, 287–306
26. Shimizu, Y., and Hunt, S. W., III (1996) Immunol. Today 17, 565–573
27. Rodriguez-Viciana, P., Varne, P. H., Vanhaesebroeck, B., Waterfield, M. D., and Downward, J. (1999) EMBO J. 18, 2442–2451
28. von Wichert, G., Haimovich, B., Feng, G. S., and Sheetz, M. P. (2003) EMBO J. 22, 5023–5035
29. Greene, D. K., Tumova, S., Couchman, J. R., and Woods, A. (2003) J. Biol. Chem. 278, 7617–7623
30. Fuchiki, K., Endo, T., Imamura, M., and Takenawa, T. (1994) J. Biol. Chem. 269, 1518–1522
31. Gillham, H., Goldberg, M. C., Pepperkok, R., and Gullick, W. J. (1999) J. Cell Biol. 146, 869–880
32. Lang, K., Doughton, R. L., Firestone, A. J., Bunce, M. W., and Anderson, R. A. (2002) Nature 420, 89–93
33. Pomies, P., Louis, H. A., and Beckerle, M. C. (1997) J. Cell Biol. 139, 157–168
34. Li, J., Yen, C., Liaw, D., Podosypina, K., Bose, S., Wang, S. I., Puc, J., Miliarets, C., Rodgers, L., McBride, R., Bigner, S., H. Giavonella, B. C., Ittmann, M., Tycko, B., Hishonho, H., Wigler, M. H., and Parsons, R. (1997) Science 275, 1943–1947
35. Li, D., and Griffith, W. S., III (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 14506–14511
36. Rodriguez-Viciana, P., Warne, P. H., Khwaja, A., Marte, B. M., Pappin, D., Das, P., Waterfield, M. D., Ridley, A., and Downward, J. (1997) Cell 89, 457–467