Ena/VASP proteins influence many actin-dependent processes in cells and developing organisms, including fibroblast and keratocyte migration (1, 2); chemotaxis by Dictostelium amoebae (3); motility of Listeria monocytogenes (4); formation of filopodia, neurite outgrowth and guidance in neurons; cell-cell adhesion; and epithelial morphogenesis in Xenopus, mice, and Drosophila (5–7). In vitro, Ena/VASP proteins bind monomeric actin and profilin-actin (8, 9), interfere with barbed end capping (10), and bundle filaments (4, 11–13), all of which could promote actin assembly.

Most biochemical studies of Ena/VASP protein regulation of actin filament dynamics examined the assembly of actin filaments in solution or of actin networks formed on nucleation-competent beads or lipid droplets. Given the diverse collection of activities exhibited by Ena/VASP proteins on actin filaments (monomer binding, nucleation, elongation bundling, and branch dissociation), interpreting the results of experiments that examine the behavior of a population of actin filaments is complex. Indeed, one study of actin polymerization in the presence of VASP and barbed end-capping proteins concluded that enhanced filament assembly in the presence of cappers resulted from the nucleation activity of VASP (14), whereas other studies carried out under conditions where filament nucleation is negligible concluded that VASP promotes filament assembly by competing with capping protein for binding barbed ends, but effects of VASP on filament elongation were not ruled out (10).

To determine how Ena/VASP proteins regulate actin dynamics at barbed ends, we used total internal reflection fluorescence (TIRF) microscopy to observe directly actin filaments growing in the presence of VASP. TIRF microscopy has been used to observe branched filament nucleation by Arp2/3 complex (15), nucleation and processive elongation by formins (16), barbed end capping (17) and uncapping (18), and filament severing by actin-depolymerizing factor/cofilin (19). We examined how VASP influences the rates of filament growth and its inhibition of barbed end capping and detected direct interactions of VASP and actin filament barbed ends. Our findings support the hypothesis that transient interactions of Ena/VASP proteins with actin filament barbed ends allow this family of proteins to regulate the rate of actin filament elongation, barbed end capping and provide a mechanism to recruit filament barbed ends during assembly of specialized actin filament structures.

**EXPERIMENTAL PROCEDURES**

**Protein Purification and Labeling**—Gel-filtered skeletal muscle actin (20) was labeled with Alexa-488 succinimidyl ester (21) or with Oregon Green iodoacetamide (22) as described; reactive fluorophores were purchased from Molecular Probes. Recombinant murine His-tagged VASP and VASP mutant proteins were prepared as described (10). A plasmid for expression in bacteria of mutant VASP-GABΔFAB was constructed by site-directed mutagenesis of the G-actin binding (GAB) motif (R232E,K233E) using a plasmid constructed for expression of VASPΔFAB as template (10). A plasmid for expression of a fusion protein of amino acids 276–374 of murine VASP fused to GST (GST-CT) was constructed using a PCR product obtained from the cDNA of murine VASP cloned into the EcoRI and BamH1 sites of pGEX-4T-1. Recombinant capping protein (α1β1) (23) and profilin (24) were prepared as described. NEM-myosin was prepared as described (25) using chicken skeletal muscle myosin purchased from Sigma.

**Total Internal Reflection Fluorescence Microscopy**—Glass coverslips were cleaned and assembled into sample chambers on glass slides as described (22). Chambers were coated for 1 min with either NEM-myosin, VASP, or control protein fol-
allowed by two washes with 1% BSA-containing buffer and once with MKEI-100 buffer (20 mM imidazole, pH 7.0, 100 mM KCl, 1 mM EGTA, 2 mM MgCl₂). TIRF microscopy was performed using an Olympus X71 inverted microscope equipped for through-the-lens total internal reflection illumination by an Argon laser; a ×60, 1.45 n.a. objective lens in combination with a ×1.5 auxiliary lens was used. Image acquisition began 30–60 s after flowing the sample into the chamber. Images (0.5-s exposure) were collected at 3- or 5-s intervals for up to 10 min by a Cool-Snap ES CCD camera (Photometrics) controlled by Isee imaging software (Innovision).

**Actin Assembly Assay**—Oregon Green (OG)-actin filament seeds were prepared from 1 μM actin (30% OG-actin) primed for 1 min with 1/8 volume of 0.8 mM EGTA, 0.08 mM MgCl₂ and added to imaging buffer (10 mM imidazole, pH 7.0, 100 mM KCl, 1 mM MgCl₂, 1 mM EGTA, 0.3% methylcellulose, 40 mM dithiothreitol, 80 μg/ml glucose oxidase, 40 μg/ml catalase, and 0.2 mM ATP). The reaction proceeded for 1 min, and a 10-μl aliquot was flowed into a 14-nM NEM-myosin-coated chamber and incubated an additional 2 min. A second reaction containing 0.75 μM actin (8% Alexa 488-actin) primed as described above was mixed with imaging buffer containing 0.4% methylcellulose with and without VASP/profilin. A 10-μl aliquot was flowed into the chamber containing the OG-actin filament seeds. Images were collected at a rate of 1 frame every 5 s for 100 frames. Filament growth rates were determined for 20–40 filaments by kymography using macros available in NIH ImageJ or by manually measuring filament lengths.

**Filament-capping Assays**—Actin filaments were labeled with two fluorophores: OG-actin to mark the pointed end and Alexa 488-actin to mark regions of filament growth in the presence of capping protein. The boundary between OG-actin and Alexa 488-actin became distinct as the OG-actin bleached during data collection. OG-actin seeds prepared as described above were added to a 14-nM NEM-myosin-coated chamber and incubated for 2 min. 0.75 μM actin (8% Alexa 488-actin), 2 nM capping protein and with or without VASP in imaging buffer containing 0.4% methylcellulose was flowed into the chamber with the OG-actin seeds; data were collected at a rate of 1 frame every 5 s for 100 frames. The length of capped filaments was measured using macros in ImageJ. The time required for capping each filament was determined from the capped filament length/rate of filament growth (obtained from identical reactions that did not contain capping protein). The fraction of capped filaments as a function of time was determined.

**Filament Capture Assay**—Flow chambers were coated for 1 min with 100 nM VASP or mutant VASP, followed by blocking twice with 1% BSA and washing once with MKEI-100. Actin filaments were assembled from 0.8 μM actin (10% Alexa 488-actin) in imaging buffer containing 0.24% methylcellulose for 2–3 min. An aliquot was flowed into the chamber, and data were collected at 1 frame/3 s for 200 frames. Interactions of all
filaments in the field with the surface were scored for the number of barbed end (BE) captures, pointed end captures, side interactions, and number of filaments not captured. Capped filaments were prepared by adding 4 nM capping protein to the reactions 30 s prior to flowing the sample into the chambers. The duration of the pause between capture and resumption of filament growth was quantified by counting the number of frames required for the brightest region at the barbed end of a captured filament to be displaced 2 pixels from the original capture site.

RESULTS

Actin Filament Capping Is Delayed by VASP—Using approaches that examine the collective behavior of a population of actin filaments, we previously showed that Ena/VASP proteins enhance actin polymerization in the presence of barbed end-capping proteins, an effect that is further augmented by profilin (10). We confirm these results by observing individual filaments elongating in the presence of VASP and capping protein (Fig. 1A). Filaments grow longer (Fig. 1C) and elongate for longer times before growth terminates by capping (Fig. 1B). These findings confirm that Ena/VASP proteins have anti-capping activity.

VASP and Profilin Enhance the Rate of Filament Elongation—Anti-capping could result from increased rates of filament elongation, by direct competition between VASP and capping protein for binding actin filament barbed ends, or by a combination of both mechanisms. To determine whether VASP enhances the rate of actin filament elongation, we measured the elongation rates of filaments growing in the presence and absence of VASP and profilin. A small fraction of the actin used in these experiments was fluorescent Alexa 488-actin, so actin filaments were labeled in a random speckled pattern that revealed the fast growing barbed end (Fig. 2A). In 0.75 μM Alexa-labeled actin, filaments grew at an average rate of 1.68 ± 0.01 μm/min (Fig. 2B). As expected from the lower rate constant for association of profilin-actin at barbed ends (26) compared with actin (27), filaments grew more slowly, 1.36 ± 0.02 or 1.16 ± 0.02 μm/min, respectively, in the presence of 2.6 or 5 μM profilin (Fig. 2B). Although VASP alone at concentrations from 12–100 nM had no effect on the rate of filament elongation (Fig. 2B and data not shown), filaments elongated 15–20% faster in the presence of profilin and VASP than with profilin-actin alone (Fig. 2B). Filament growth in the presence of VASP and profilin never exceeded the rates observed with actin alone. Thus, anti-capping activity by VASP alone does not result because filaments elongate more quickly. However, VASP anti-capping is enhanced in the presence of profilin-actin, in part by increasing the rate of filament growth.

Barbed End Capture by VASP—To examine whether Ena/VASP proteins associate directly with actin filament barbed ends, we developed a TIRF microscopy-based assay. Short, elongating actin filaments were flowed into a chamber coated with 100 nM VASP. Filaments initially diffused freely within the evanescent field. Gradually, filaments stopped diffusing and became tethered at the surface via attachment of their barbed ends (Fig. 3A and supplemental Movie S1). Once captured, end-attached filaments paused briefly but resumed growing within four video frames (~12 s). Captured filaments remained bound to the surface via an interaction along the side of the filament at the site where the initial end capture had occurred. The barbed...
end of captured filaments was frequently captured again at other sites on the surface as the filament continued to elongate. On average, filaments experienced $1.45 \pm 0.24$ BE captures/filament compared with $0.32 \pm 0.10$ on control, BSA-coated surfaces (Table 1). By 10 min, ~92% of filaments in the microscope field had been captured by VASP-coated surfaces compared with only ~42% of filaments in the presence of BSA-coated surfaces (Table 1). Filament capture by BSA-coated surfaces was often transient and filaments detached from the surface, whereas those captured by VASP-coated surfaces remained bound for the duration of the movie (supplementary Movie S2). Capture of filaments via an interaction with the side of filaments was infrequent ($0.15 \pm 0.04$ side capture/filament; Table 1), and capture via the slow growing, pointed end was rarely observed ($0.015 \pm 0.008$ pointed end capture/filament; Table 1). Capped filaments were not captured, indicating that barbed end capture by VASP required free, growing barbed ends (Fig. 3B). Consistent with this result, non-growing filaments that were occasionally observed in reactions without capping protein were never captured by VASP-coated surfaces. Profilin increased the efficiency of barbed end capture by VASP ~1.6-fold (Fig. 3B).

Two observations support the notion that growing filaments preferentially interact with VASP-coated surfaces via their barbed ends rather than via the side of newly polymerized segments. First, capped filaments were not captured. Second, filament growth ceased for several seconds immediately after capture (Table 2), suggesting that interaction with VASP on the glass surface transiently blocked actin monomer addition. To further distinguish between side versus end capture events, we examined the behavior of filaments interacting with immobilized cortactin, which binds the sides of actin filaments (28, 29). Some filaments associated with cortactin-coated surfaces via interactions scored as barbed end capture events ($0.9$ BE captures/filament). However, in contrast with VASP-coated surfaces where few filaments were captured via their sides, the majority of filaments captured by cortactin-coated surfaces were via side interactions ($2.5$ side captures/filament). Thus, actin filaments preferentially interact with VASP at their barbed end.

How Does VASP Interact with the Barbed Ends of Actin Filaments?—Ena/VASP proteins interact with actin monomer and filaments through GAB and F-actin binding motifs (FAB), respectively, situated within the C-terminal EVH2 domain (12, 30). Each of these motifs is essential for anticapping activity (10). Filament capture via the GAB or FAB motifs, which also resemble WH2 motifs, was predicted from recent findings that a WH2 motif of N-WASP captures actin filament barbed ends (31). To determine whether the VASP GAB or FAB motifs were involved in barbed end capture, we observed filaments growing above surfaces.

![FIGURE 3. VASP captures actin filament barbed ends. A, time lapse series showing capture of filaments by a VASP-coated surface using TIRF microscopy. The entire video sequence is included in the supplemental data section (supplemental Movie S1). At early times, filaments freely diffuse. Arrowheads mark the site of initial filament end capture by a site on the coverslip surface, which occurred in this sequence at frame 54. Growth of the end-bound filament pauses for several frames (through frame 57) concomitant with transition to filament-side interaction. The invariant speckle pattern along the older portions of the filament confirms that the captured filament is stably bound to the surface and not freely diffusing. Numbers in each panel indicate the frame number from the movie (1 frame/3 s). B, relative barbed end capture efficiency by VASP- and VASP mutant-coated surfaces (VASP-GAB, VASP-ΔFAB, and VASP-GAB-ΔFAB). Chambers were coated with 100 nM VASP or VASP mutant proteins and blocked with 1% BSA. Control chambers were treated with the VASP buffer prior to blocking. Actin filaments were flowed into the chambers, and the number of BE capture events were quantified. In experiments with 2.5 μM profilin, it was added to filaments just before flowing in the chambers. In experiments with capped filaments, filaments were treated for 30 s with 4 nM capping protein before flowing into a chamber. Error bars indicate S.E.](Image 221x26 to 248x38)

### TABLE 1

| Surface coating | Total filaments | BE capture/filament | Side capture/filament | PE capture/filament | % Non-attached |
|-----------------|-----------------|---------------------|-----------------------|---------------------|---------------|
| 1% BSA          | 221             | 0.32 ± 0.10         | 0.14 ± 0.03           | 0.008 ± 0.008       | 57 ± 7.8%     |
| 100 nM VASP     | 258             | 1.45 ± 0.24         | 0.15 ± 0.04           | 0.015 ± 0.008       | 7.8 ± 2.5%    |

Interactions of actin filaments with VASP-coated and BSA-coated surfaces

BE, barbed end; PE, pointed end. Data compiled from six independent experiments.
coated with mutant proteins carrying either a mutated GAB motif (VASP-GAB; R232E,K233E) lacking the FAB motif (VASP-ΔFAB) or a double mutant protein (VASP-GAB-ΔFAB) disrupted at both motifs. Surprisingly, barbed end capture by all three mutant VASP proteins was as efficient as with wild-type VASP (Fig. 3B). Filaments captured by mutant VASP proteins transitioned to a side interaction and resumed growing, as observed with wild-type VASP. However, the pause in growth exhibited by captured filaments during the transition from end-to-side attachment was shorter for filaments captured by mutant VASP proteins (Table 2). Thus, although the GAB and FAB motifs are required for anti-capping activity, they are not required for interaction of VASP with barbed ends.

The C Terminus of VASP Captures Barbed Ends—To identify a region within VASP capable of binding barbed ends, we focused on the EVH2 domain and tested a fragment of VASP, from distal to the FAB motif through the C terminus and fused to glutathione S-transferase (GST-CT; amino acids 276–374 of murine VASP). Filaments were captured by GST-CT-coated surfaces 2.5 ± 0.6-fold more efficiently than by GST-coated surfaces (supplemental Movies S3 and S4). Whereas 45% of filaments remained freely diffusing near GST-coated surfaces, only 15% of filaments did not bind GST-CT-coated surfaces. Filaments captured by GST-CT were more likely to dissociate from the surface (~14% transient captures by GST-CT compared with <2% for VASP). Filament capture via the sides of filaments was slightly higher with GST-CT (~0.3 side captures/filament) than with VASP (~0.15 side captures/filament). The pause between end capture and resumption of growth was similar to that observed for filaments captured by the doubly mutant protein, VASP-GABΔFAB (Table 2). GST-CT alone neither exhibited capping activity nor interfered with VASP anti-capping activity when examined in solution assays with pyrenyl-actin (supplemental Fig. S1). GST-CT also did not bind actin filaments subjected to high speed pelleting (data not shown).

### DISCUSSION

Our observations reveal two features of the mechanism by which Ena/VASP proteins modulate actin dynamics and filament organization. First, filaments growing in the presence of VASP and profilin-actin elongate faster than filaments growing in the presence of profilin-actin alone. Second, VASP associates directly with actin filament barbed ends and profilin increases the efficiency of barbed end capture by VASP. These findings support the hypothesis that transient interactions of Ena/VASP proteins with actin filament barbed ends allow this family of proteins to regulate the rate of actin filament elongation, barbed end capping and provide a mechanism to recruit filament barbed ends during assembly of specialized actin filament structures.

Several features of the interaction of VASP and actin filaments were unexpected. First, filament side attachments were infrequent, even with very long filaments. Thus, the potent F-actin binding and bundling activities of VASP detected in vitro (10, 12, 32) likely initiate from interactions of VASP with barbed ends. Because Ena/VASP proteins are generally situated at structures in vivo enriched with actin filament barbed ends, a mechanism that promotes rapid dissociation of Ena/VASP from the sides of actin filaments must exist. Phosphorylation may regulate the association of VASP with the sides of actin filaments in vivo, because protein kinase A-treated VASP did not bundle filaments in vitro (10). Second, although anti-capping activity required functional GAB and FAB (10), barbed end capture did not. This was unexpected because the WH2 motif of N-WASP, which shares homology with GAB and FAB, was proposed to maintain the attachments of actin filaments in comet tails propelling glass beads via interactions with filament barbed ends (31). We found that a region at the C terminus between amino acids 276 and 374, downstream of FAB, is sufficient to capture barbed ends, but GAB and FAB likely stabilize this interaction to effect anti-capping. The longest pause between the time of end capture and resumption of growth occurred with wild-type VASP and the shortest occurred with VASP-GABΔFAB or GST-CT, both of which lack functional GAB and FAB motifs. Thus, the GAB and FAB motifs, together with profilin, may serve to orient VASP at barbed ends to block interactions with capping proteins or to organize an array of several filaments bound to a single VASP molecule.

Finally, our expectation that growing filaments might associate continuously via their barbed ends to VASP-coated surfaces, as observed with some formins (33–35), was not realized. Processive association of Ena/VASP with growing filament barbed ends has been suggested from theoretical considerations (36), from structural studies (9, 37), and from recent fluorescence recovery after photobleaching experiments of GFP-VASP at the tips of growing filopodia (38). Although observing the dynamics of single actin filaments by TIRF microscopy offers unique advantages, there are some limitations of this approach. In our assay, immobilized VASP is rigidly attached to the coverslip surface and is only tickled by single filaments. If processive association of Ena/VASP proteins with elongating actin filaments requires a flexible VASP tetramer associated with two (or more) filaments, as has been suggested by some models (9, 37), such events would not be readily detected. Filaments diffusing over surfaces coated with a VASP binding fragment of the *Listeria* ActA protein in the presence of soluble VASP also exhibited transient barbed end capture.3 Although we observed barbed end-attached filaments that elongated while remaining attached via their ends on rare occasions, we cannot be certain these events resulted from

---

3 D. A. Schafer, unpublished results.
Actin Filament Capture by VASP

**bona fide** processive interactions of VASP with barbed ends or from random, nonspecific interactions with the protein-coated surface. Nonetheless, the ability of VASP-coated surfaces to capture filaments via their barbed ends suggests that Ena/VASP proteins function to recruit filaments of the lamellipodial network and transiently block capping during the formation of nascent protrusions.

Acknowledgment—We thank Dr. Ammasi Periasamy of the Keck Imaging Center at the University of Virginia for assistance with the TIRF microscope.

**REFERENCES**

1. Bear, J. E., Loureiro, J. J., Libova, I., Fassler, R., Wehland, J., and Gertler, F. B. (2000) *Cell* **101**, 717–728
2. Lacayo, C. I., Pincus, Z., VanDuijn, M. M., Wilson, C. A., Fletcher, D. A., Gertler, F. B., Mogilner, A., and Theriot, J. A. (2007) *PLoS Biol.* **5**, e233
3. Han, Y. H., Chung, C. Y., Wessels, D., Stephens, S., Titus, M. A., Soll, D. R., and Firtel, R. A. (2002) *J. Biol. Chem.* **277**, 49877–49887
4. Laurent, V., Wiersma, D., Herve, F., and Faix, J. (2003) *J. Biol. Chem.* **278**, 28653–28662
5. Kwiatkowski, A. V., Rubinson, D. A., Dent, E. W., Edward van Veen, J., Mahaffy, R. E., and Pollard, T. D. (2006) *J. Biol. Chem.* **281**, 1245–1258
6. Chereau, D., and Dominguez, R. (2006) *Biochemistry* **45**, 195–201
7. Barzik, M., Kotova, T. I., Higgs, H. N., Hazelwood, L., Hanein, D., Gertler, F. B., and Schafer, D. A. (2005) *J. Biol. Chem.* **280**, 28653–28662
8. Kuhn, J. R., and Pollard, T. D. (2007) *J. Biol. Chem.* **282**, 28014–28024
9. Kim, K., McCully, M. E., Bhattacharya, N., Butler, B., Sept, D., and Cooper, J. A. (2007) *J. Biol. Chem.* **282**, 5871–5879
10. Kragtorp, K. A., and Miller, J. R. (2006) *Development* **133**, 685–695
11. Gates, J., Mahaffey, R., Rogers, S. L., Emerson, M., Rogers, E. M., Sottile, S. L., Van Vactor, D., Gertler, F. B., and Peifer, M. (2007) *Development* **134**, 2027–2039
12. Kuhn, J. R., and Pollard, T. D. (2007) *J. Biol. Chem.* **282**, 1245–1258
13. Ferron, F., Rebowski, G., Lee, S. H., and Dominguez, R. (2007) *FEBS Lett.* **5871–5879
14. Ferron, F., Rebowski, G., Lee, S. H., and Dominguez, R. (2007) *PLoS Biol.* **5**, e233
15. Barzik, M., Kotova, T. I., Higgs, H. N., Hazelwood, L., Hanein, D., Gertler, F. B., and Schafer, D. A. (2005) *J. Biol. Chem.* **280**, 28653–28662
16. Michielot, A., Berro, J., Guepin, C., Boujemaa-Paterski, R., Staiger, C. J., Martiel, J. L., and Blanchon, L. (2007) *Curr. Biol.* **17**, 825–833
17. Mahaffy, R. E., and Pollard, T. D. (2006) *Biophys. J.* **91**, 3519–3528
18. Walders-Harbeck, B., Khaitlina, S. Y., Hinssen, H., Jockusch, B. M., Wehland, J., Gertler, F. B., and Carlier, M. F. (1999) *J. Cell Biol.* **144**, 1245–1258
19. Mahaffy, R. E., and Pollard, T. D. (2005) *Biophys. J.* **88**, 1387–1402
20. Lu, J., and Pollard, T. D. (2001) *Mol. Biol. Cell* **12**, 1161–1175
21. Kuhn, J. R., and Pollard, T. D. (2005) *J. Cell Biol.* **169**, 875–887
22. Veigel, C., Bartoo, M. L., White, D. C., Sparrow, J. C., and Molloy, J. E. (1998) *Biophys. J.* **75**, 1424–1438
23. Gutsche-Perelroizen, I., Lepault, J., Ott, A., and Carlier, M. F. (1999) *J. Biol. Chem.* **274**, 6234–6243
24. Wolters-Harbeck, B., Khaitlina, S. Y., Hinssen, H., Jockusch, B. M., and Illenberger, S. (2002) *J. Biol. Chem.* **277**, 28014–28024
25. Kuhn, J. R., and Pollard, T. D. (2000) *J. Cell Biol.* **150**, 29–40
26. Gutsche-Perelroizen, I., Lepault, J., Ott, A., and Carlier, M. F. (1999) *J. Biol. Chem.* **274**, 6234–6243
27. Pollard, T. D. (1986) *J. Cell Biol.* **103**, 2747–2754
28. Wu, H., and Parsons, J. T. (1993) *J. Cell Biol.* **120**, 1417–1426
29. Weed, S. A., Karginov, A. V., Schafer, D. A., Weaver, A. M., Kinley, A. W., Cooper, J. A., and Parsons, J. T. (2000) *J. Cell Biol.* **151**, 29–40
30. Kuhn, J. R., and Pollard, T. D. (2005) *J. Cell Biol.* **169**, 875–887
31. Ferron, F., Rebowski, G., Lee, S. H., and Dominguez, R. (2007) *J. Biol. Chem.* **282**, 14725–14730
32. Kuhn, J. R., and Pollard, T. D. (2007) *J. Biol. Chem.* **282**, 28014–28024
33. Barzik, M., Kotova, T. I., Higgs, H. N., Hazenwood, L., Hanein, D., Gertler, F. B., and Schafer, D. A. (2005) *J. Biol. Chem.* **280**, 28653–28662
34. Kuhn, J. R., and Pollard, T. D. (2007) *J. Biol. Chem.* **282**, 28014–28024
35. Amann, K. J., and Pollard, T. D. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 15009–15013
36. Barzik, M., Kotova, T. I., Higgs, H. N., Hazelwood, L., Hanein, D., Gertler, F. B., and Schafer, D. A. (2005) *J. Biol. Chem.* **280**, 28653–28662
37. Barzik, M., Kotova, T. I., Higgs, H. N., Hazelwood, L., Hanein, D., Gertler, F. B., and Schafer, D. A. (2005) *J. Biol. Chem.* **280**, 28653–28662
38. Applewhite, D. A., Barzik, M., Kojima, S., Svitkina, T. M., Gertler, F. B., and Borisy, G. G. (2007) *Mol. Biol. Cell* **18**, 2579–2591