Cytoskeletal dynamics are important for efficient function of the secretory pathway. ADP-ribosylation factor, ARF1, triggers vesicle coat assembly and, in concert with Cdc42, regulates actin polymerization and molecular motor-based motility. Drebrin and mammalian Abp1 (mAbp1) are actin-binding proteins found previously to bind to Golgi membranes in an ARF1-dependent manner in vitro. Despite sharing homology through two shared actin binding domains, drebrin and mAbp1 have different subcellular localization and bind to distinct actin structures on the Golgi apparatus. We find that the actin-depolymerizing factor homology (ADFH) and charged/helical actin binding domains of drebrin and mAbp1 are sufficient for regulated binding to Golgi membranes and subcellular localization. We have used mutant proteins and chimeras between mAbp1 and drebrin to identify motifs that direct targeting. We find that a linker region between the ADFH and charged/helical domains confers Golgi binding properties to mAbp1. mAbp1 binds to a specific actin pool through its ADFH/linker domain that is not bound by drebrin. Drebrin localization to the cell surface was found to involve motifs within the charged/helical domain. Our results indicate that targeting of these proteins is directed through multiple distinct interactions with the actin cytoskeleton. The mechanisms for selective recruitment of mAbp1 and drebrin to Golgi membranes indicate how actin-based structures are able to select specific actin-binding proteins and, thus, carry out multiple different functions within cells.

Efficient utilization of vesicular transport within the secretory pathway requires coordinated regulation of cargo-protein sorting, vesicle coat assembly, vesicle fission, cytoskeleton-mediated translocation, and membrane fusion (1). For example, cytoskeleton and molecular motor-based motility must be connected to vesicle formation to ensure that vesicles are not translocated prematurely or belatedly. Previous studies show that the vesicle coat proteins can influence actin dynamics and molecular motor function at the Golgi complex (2).

The actin cytoskeleton is linked to trafficking in the early secretory pathway (3–7). Two small GTP-binding proteins, ARF1 and Cdc42, function at the interface between vesicle trafficking at the Golgi complex and cytoskeletal regulation (5, 6, 8–11). ARF isoforms may regulate actin dynamics at multiple sites in the cell (12–14). Active ARF1 causes the recruitment of Cdc42 to Golgi membranes through its binding interaction with the γ-subunit of coatomer, the coat proteome 1 (COPI) vesicle coat protein (5, 15). ARF1-dependent Cdc42 on Golgi membranes is further regulated by a specific GTPase-activating protein (GAP) protein, ARHGAP10 (8). Active Cdc42 stimulates Arp2/3-mediated actin polymerization at the Golgi complex (3) and inhibits the binding of the microtubule motor protein dynein to COPI vesicles (16). Importantly, the binding interaction between coatomer and Cdc42 is inhibited in the presence of the C-terminal coatomer binding motif of a putative cargo receptor protein, p23 (5, 15). Thus, actin dynamics and dynein recruitment on Golgi membranes are potentially regulated by both vesicle coat assembly and cargo protein packaging. This regulation may ensure that vesicle translocation occurs only after vesicle formation is completed.

We have found that the actin-binding proteins mAbp1, drebrin, and cortactin are recruited to Golgi membranes upon activating ARF1 (5, 6, 17). Their recruitment appears to be specific since other cytosolic actin-binding proteins are not ARF1-dependent (6). Drebrin and mAbp1 are homologous to the yeast actin-binding protein, ABP1, which functions during endocytosis (18–20). In mammalian cells mAbp1 has been implicated in endocytosis and in Golgi trafficking (5, 21). mAbp1 is localized to the plasma membrane and to the juxtanuclear region of cells. During endocytosis, mAbp1 binds to dynamin, a GTP-binding protein involved in vesicle fission, through a C-terminal Src homology domain 3 domain (21). Drebrin, by contrast, is found mostly at the cell surface and is implicated in GTPase-activating protein junction assembly (22) and neurite development (23). Overexpression of drebrin leads to the dramatic increase in filopodia-like cell-surface protrusions (24, 25).

Drebrin and mAbp1 are homologous through two actin binding domains present at their N termini. An actin-depolymerizing factor homology (ADFH) domain is present at the N terminus of both proteins. The crystal structure for yeast ABP1 ADFH domain has recently been reported and confirms the structural similarity with other ADFH domain-containing proteins (26). The second actin binding domain is a charged/helical motif that appears to be specific to just these two proteins. Motifs within the charged/helical domain of drebrin have been found to be important for its subcellular localization (24).

Remarkably, although drebrin and mAbp1 share sequence homology and are both responsive to ARF1, they appear to be recruited to distinct actin-based structures on Golgi membranes in vitro (5). Drebrin is present with an actin pool that can be extracted with salt, and its binding is sensitive to the actin toxin, cytochalasin D. By contrast, mAbp1 is resistant to salt extraction and binds to Golgi membranes in the presence of cytochalasin D. Unlike drebrin, mAbp1 is sensitive to disrupting the coatomer–Cdc42 complex with a peptide corresponding to 18 U.S.C. Section 1734 solely to indicate this fact.
to the coatomer binding motif of p23. These findings suggest that mAbp1 is recruited to actin-based structures formed through the action of the coatomer/Cdc42 complex, whereas drebrin is recruited through an alternative mechanism.

The mechanisms for recruiting F-actin-binding proteins to distinct subcellular regions and to specific actin-based structures are poorly understood (27). Here we have investigated sequence motifs on drebrin and mAbp1 that confer their common and distinct properties. We find that the actin binding domains are sufficient for regulated recruitment to membranes. However, these conserved domains have different properties that direct mAbp1 and drebrin to specific actin-based structures.

**EXPERIMENTAL PROCEDURES**

**Materials**—Rat liver Golgi membranes and bovine brain cytosol were isolated as described previously (28). The following antibodies were used: mouse anti-giantin (Berkeley Antibody Co., Inc.), rabbit anti-giantin (Covance), anti-drebrin (MBL International Corp.), anti-β-COP (Sigma). Goat anti-GST was a gift from R. Piper. Rhodamine phalloidin was obtained from (Cytoskeleton Inc.). All other antibodies and materials were obtained as described previously (5, 6).

**Constructing Plasmids for the Expression of mAbp1 and Drebrin**—Original mAbp1 construct in pRK5 vector was a gift from M. Kessels. Drebrin E cDNA was amplified by PCR from a rat cDNA library and inserted into pGEM-T easy vector. The cDNA fragments encoding the different truncations and chimeras of mAbp1 and drebrin were obtained by PCR. Chimeras were generated using consecutive PCR reactions. The first PCR reactions utilized an appropriate chimeric oligonucleotide. The product from the first reaction was then used as a primer for the second reaction to complete a cDNA encoding the desired chimeric protein. Proline point mutations were introduced by PCR using the QuikChange mutagenesis kit (Stratagene). The sequences for the oligonucleotide primers used in this study are available upon request.

To express the truncated, chimeric, and full-length drebrin and mAbp1 as GST fusion proteins, they were cloned into the bacterial expression vector pGEX-4T-2. After induction, the expressed proteins were purified using glutathione-Sepharose as described previously (5, 6). The protein concentrations were estimated by the absorbance at 280 nm. The encoding cDNAs were also ligated into pEGF-C1 or pEGFP-N1 plasmids for expression as GFP fusion proteins in Vero cells.

**Golgi Membrane Binding Assays**—Golgi membrane binding and budding reactions were carried out as described previously (3, 5, 6, 29). The following concentrations were used during the incubations: GTPγS (20 μM), cytochalasin D (20 μM), latrunculin A (2 μM), p23 peptide (250 μM), and brefeldin A (400 μM). The recombinant protein concentration was ~0.01 mg/ml. The Golgi membranes were reisolated from the reaction by flotation (5, 6, 29). Western blot signals were quantified by densitometry where indicated.

**F-Actin Binding Assay**—Phalloidin-dependent F-actin binding assays were performed to confirm that the truncated and chimeric proteins were folded properly with active actin binding motifs (Supplemental Fig. 1). The assays were conducted using the same conditions as the Golgi membrane binding assays except that the GTPγS and membranes were omitted. The final reaction volume was 0.5 ml, and the protein concentration was ~0.01 mg/ml. Phalloidin (20 μM) was added to the reaction before the incubation where indicated. The polymerized actin and bound proteins were isolated by sedimentation, solubilized in Laemmli sample buffer, and subjected to Western blot analysis.

**RESULTS**

**Recombinant Drebrin and mAbp1 Bind Golgi Membranes in a Regulated Manner**—We have used a cell-free binding assay to characterize motifs that are important for the recruitment of mAbp1 and drebrin to the Golgi membranes. We first tested whether recombinant full-length mAbp1 and drebrin bind to membranes with properties similar to cytosol-derived proteins. GST-mAbp1 and GST-drebrin bind to rat liver Golgi membranes when GTP-binding proteins are activated by the non-hydrolyzable GTP analog, GTPγS (Fig. 1). Like cytosolic drebrin (5, 6), GST-drebrin binding is sensitive to cytochalasin D (Fig. 1, A and B). Disrupting coatomer/Cdc42 signaling using a peptide corresponding to the C-terminal coatomer binding domain of p23 had little effect on GST-drebrin binding. By contrast, GST-mAbp1 binding was sensitive to the p23 peptide and insensitive to cytochalasin D (Fig. 1, C and D). The binding of the coatomer subunit, β-COP, is unaffected by cytochalasin D or the peptide, indicating that COPII vesicle coat assembly does not require regulated actin dynamics. GST-drebrin, GST-mAbp1, β-COP, and actin levels were all sensitive to brefeldin A, confirming that they are induced by ARF activation (Fig. 1, A and C). These results show that regulated binding properties of GST-drebrin and GST-mAbp1 are the same as those reported previously for the endogenous cytosolic proteins. We conclude that the recombinant GST-tagged proteins will be useful for defining the domains that confer selective binding.

**The N-terminal Actin Binding Domains Are Sufficient for Binding to the Golgi**—The conserved ADFH and charged/helical actin binding domains are present in the N-terminal region of mAbp1 and drebrin (Fig. 2A). The C-terminal portions of drebrin and mAbp1 are poorly conserved, and mAbp1 contains a C-terminal Src homology domain 3 that is absent from drebrin. We anticipated that the different binding
Targeting Motifs on Drebrin and mAbp1

**A**

![Diagram showing binding domains of mAbp1 and drebrin.](Image)

**B**

| mAbp1/SH3p7 | ADFH | CH | PP | SH3 |
|-------------|------|----|----|-----|
| M. musculus | 123  | 171|     |     |
| R. norvegicus | 124 | 178|     |     |

**C**

| mAbp1(1-170) | Drebrin (1-270) |
|--------------|-----------------|
| GTP-S        | β-cop          |
| CytoD        | actin          |
| p23 peptide  |                 |
| GTP-S        | β-cop          |
| CytoD        | actin          |
| p23 peptide  |                 |

**FIGURE 2.** The N-terminal actin binding domains of drebrin and mAbp1 are sufficient for the regulated recruitment to Golgi membranes. A, a diagram comparing the domain structure of mAbp1 and drebrin. The ADFH, charged/helical (CH), proline-rich (PP), flexible helix (FH), Homer ligand (H), Src homology domain 3 (SH3), and SH2 ligand (SL) domains are indicated. M. musculus, R. norvegicus. B and C, Golgi binding assays were used to characterize the recruitment of truncated forms of mAbp1 (B) and drebrin (C) as in Fig. 1. GTP·S, cytoskeleton; and the p23 C-terminal peptide were added to the incubations as indicated. The construct numbers as listed in Table 1 are 2 (B) and 11 (C).

and localization properties of these proteins could be conferred by unique C-terminal motifs. We tested this by characterizing the binding of truncated mAbp1 (Fig. 2B) and drebrin (Fig. 2C), containing only the N-terminal conserved actin binding region (2 and 11 in Table 1). The truncated proteins bound the Golgi membranes in a regulated manner identical to that we had observed for the full-length endogenous proteins (compare Figs. 1 and 2). We confirmed that the N-terminal domains were sufficient to direct Golgi binding by constructing chimeric proteins between drebrin and mAbp1. A chimera (5 in Table 1) containing the N-terminal actin binding domains from mAbp1 and the unique C-terminal motif from drebrin bound Golgi membranes in a GTP·S-dependent manner just like mAbp1 (Supplemental Fig. 2A). The chimeric protein containing the N terminus from drebrin and the C terminus from mAbp1 (14 in Table 1) bound in a cytoskeleton D-sensitive manner like drebrin (Supplemental Fig. 2B). The chimeric proteins bound to Golgi membranes at roughly the same levels as wild-type full-length mAbp1 and drebrin (Supplemental Fig. 2C). Together, these results show that the distinct binding properties are not conferred by the unique C-terminal regions of mAbp1 and drebrin but instead involve the more conserved N-terminal actin binding domains.

A Region between the AD FH and the Charged/Helical Domains Is Involved in Regulated Binding—Because drebrin and mAbp1 have two previously defined actin binding domains, we tested which of these might be responsible for conferring the membrane binding properties. We constructed a chimera in which the AD FH domain from mAbp1 was fused to the charged/helical domain from drebrin (8 in Table 1). The binding of this chimera was partially sensitive to both cytoskeleton D and disrupting Cdc42 with the p23 peptide (Table 1). Thus, the chimeric protein seemed to have binding properties intermediate between mAbp1 and drebrin. The result suggested that neither the AD FH domain nor the charged/helical region was fully sufficient to confer properly regulated binding to the Golgi membranes. Some chimeric proteins containing the drebrin AD FH domain and mAbp1 charged/helical domain were inactive in the binding assays and, thus, were uninformative (16, 17, and 19 in Table 1).

We noted that the region between the AD FH and charged/helical domains (mAbp1 residues 123–171) contains a stretch of 34 residues (residues 123–156) that is poorly conserved between mAbp1 and drebrin (Fig. 3A). The sequence divergence could indicate that this “linker” region is not important for protein structure or function. Alternatively, the distinct primary structure could have evolved to confer unique functional properties to mAbp1 and drebrin. To test between these possibilities, we characterized additional chimeric proteins between the mAbp1 and drebrin N termini. One chimera containing residues 1–171 of mAbp1 fused to 173–278 of drebrin (7 in Table 1) was resistant to cytoskeleton D and sensitive to the p23 peptide (Fig. 3B). This chimera contained the mAbp1 linker domain and, importantly, had binding properties similar to mAbp1. By contrast, a chimera (9 in Table 1) containing residues 1–122 of mAbp1 fused to 124–278 of drebrin and containing the drebrin linker domain was sensitive to cytoskeleton D and more resistant to the peptide, thus, behaving like drebrin. This result indicates that residues 123–171 do not comprise a passive linker region between the two actin binding domains of drebrin and mAbp1. Instead, this region appears to be a distinct motif that confers specific binding properties to these proteins.

mAbp1 Binds to a Cytoskeleton D-dependent Actin Pool via the AD FH and Linker Domains—Although the AD FH domains of drebrin and mAbp1 alone bound to F-actin (Supplemental Fig. 1), they did not bind to Golgi membranes. As an alternative approach to test the specific properties of the AD FH and linker domains, we inserted helix-breaking proline mutations in the charged/helical domains. Substituting proline at positions 189 (Fig. 4A) and 199 and 209 (not shown) of drebrin abolished all binding activity, confirming the essential role for this domain.

Proline mutations at the corresponding positions in mAbp1 also inhibited its GTP·S-dependent binding to the Golgi membranes (Fig. 4B and data not shown). Remarkably, we observed that binding of the mutant mAbp1 was restored in the presence of cytoskeleton D. We confirmed that the cytoskeleton D-dependent binding requires actin by adding both cytoskeleton D and the actin monomer binding toxin latrunculin A (Fig. 4C). This result indicates that mAbp1 can bind to a cytoskeleton D-dependent actin pool through its AD FH/linker domain but requires a functional charged/helical domain for binding to a GTP·S-dependent pool. Thus, the two actin binding domains of mAbp1 have the ability to independently direct this protein to two distinct actin-based structures. Together, the results show that the ARF1-dependent binding of both mAbp1 and drebrin requires a functional charged/helical domain.

The N-terminal Actin Binding Domains Direct Subcellular Localization—We expected that the motifs involved in regulated binding of drebrin and mAbp1 to Golgi membranes in vitro would also be important for the subcellular distribution of these proteins. Expression of recombinant drebrin and mAbp1 has been used previously to characterize subcellular localization as well as the cellular consequences of expression (21, 24, 30). We confirmed that GFP-mAbp1 localizes to the cell surface and to a juxtanuclear region near the Golgi complex (Fig. 5A). This localization is similar to that described previously for the endogenous protein (5, 30). GFP-drebrin appears predominantly localized to the cell surface and stress fibers. As in previous reports (23, 24), drebrin expression caused an increase in filopodia-like cell-surface processes (Fig. 5B).

We tested whether the N-terminal actin binding domains directed localization in cells. The chimera (14 in Table 1) containing the actin binding domains of drebrin fused to the C terminus of mAbp1 is localized to the cell surface, showing that most subcellular targeting information for drebrin is contained within the actin binding domains (Fig. 5C). Truncated drebrin (1–278), containing only the N-terminal actin binding domains, is also localized to the cell surface and induces filopodia formation (Fig. 5C). The results confirm previous studies (24)
### TABLE 1

**Truncated and chimeric forms of mAb1 and drebrin used for this study**

The numbers in the left-hand column are used to identify truncated and chimeric proteins within the text. For the membrane binding results, -- indicates <20%, --/+ indicates 20–60%, and ++ indicates >60% of the positive control value. For the cell-surface localization and filopodia formation, cells from at least two independent experiments were viewed blind and scored as plus or minus.

| mAb1 | drebrin | GTPγS dependance | CytoD sensitivity | p23-peptide sensitivity | Cell surface localization/ filopodia |
|------|---------|-----------------|-------------------|-------------------------|-------------------------------------|
| 1    |         |                 |                   |                         |                                     |
| 2    |         |                 |                   |                         |                                     |
| 3    |         |                 |                   |                         |                                     |
| 4    |         |                 |                   |                         |                                     |
| 5    |         |                 |                   |                         |                                     |
| 6    |         |                 |                   |                         |                                     |
| 7    |         |                 |                   |                         |                                     |
| 8    |         |                 |                   |                         |                                     |
| 9    |         |                 |                   |                         |                                     |
| 10   |         |                 |                   |                         |                                     |
| 11   |         |                 |                   |                         |                                     |
| 12   |         |                 |                   |                         |                                     |
| 13   |         |                 |                   |                         |                                     |
| 14   |         |                 |                   |                         |                                     |
| 15   |         |                 |                   |                         |                                     |
| 16   |         |                 |                   |                         |                                     |
| 17   |         |                 |                   |                         |                                     |
| 18   |         |                 |                   |                         |                                     |
| 19   |         |                 |                   |                         |                                     |
showing that the actin binding domains of drebrin are sufficient for both cell-surface localization and for inducing filopodia.

The chimeric protein (5 in Table 1) with the N terminus of mAbp1 fused to the C terminus of drebrin localized predominantly to cytoplasmic structures (Fig. 5D). Similar localization was observed with the truncated mAbp1 containing just the N-terminal ADFH and charged/helical domains (Fig. 5D). Some cells expressing truncated mAbp1 or the chimera (5 in Table 1) displayed distinct juxtanuclear Golgi-like localization (Fig. 5D), but juxtanuclear localization was more pronounced with the full-length protein, suggesting that C-terminal motifs may make some contribution to the subcellular distribution of mAbp1 (i.e. see Ref. 21). Importantly, the truncated mAbp1 and the chimera (5 in Table 1) did not cause an increase in filopodia or any other overt changes in cell-surface morphology.

A Motif within the Charged/Helical Domain Directs the Cell-surface Localization of Drebrin—To begin characterizing the relative contributions of the ADFH and charged/helical domains to subcellular localization, we examined the effects of disrupting the helical domain with the proline mutations. The proline substitutions had only subtle effects on the distribution of mAbp1 in cells (not shown). By contrast, the localization of drebrin to phalloidin-stained actin was greatly reduced when the helical domain was disrupted (Fig. 6A). The proline substitution also prevented drebrin from inducing filopodia formation. This result is consistent with our findings from the binding assay (Fig. 4A), indicating that the charged/helical domain is especially important for drebrin targeting.

To identify sequence motifs within the charged/helical domain, we have characterized additional truncation mutants and chimeras. Chimeras between drebrin and mAbp1 demonstrate that the charged/helical domain of drebrin is sufficient for localization to the cell surface and for inducing filopodia regardless of which ADFH or linker domain it is fused to (Table 1, 7, and 11). The C-terminal portion of the charged/helical domains (mAbp1 227–270, drebrin 228–278) did not appear necessary for binding or subcellular localization (Table 1, 3, 6, 12, and 15). Interestingly, we found that a portion of the charged/helical domain, residues 173–227, was sufficient for determining localization to the cell surface. This region from drebrin when replacing the corresponding residues of mAbp1 (18 in Table 1) directed the localization of the chimeric protein to the plasma membrane and induced filopodia (Fig. 6B). By contrast, residues 172–226 from mAbp1, when fused to
Targeting Motifs on Drebrin and mAbp1

![Image of a confocal micrograph showing GFP-drebrin and mAbp1 localization](image)

Drebrin (19 in Table 1), did not support localization to the cell-surface cortical actin (Fig. 6B). Despite specifying cell-surface localization, this charged/helical motif of drebrin did not affect Golgi binding when fused to mAbp1 (Supplemental Fig. 2). This is consistent with the notion that the Golgi binding properties are predominantly conferred by the ADFH/linker domains.

**mAbp1 Binds to Cytochalasin D-dependent Actin in Whole Cells**—Because the linker region between the ADFH and charged/helical domains appeared important for directing the binding of mAbp1 to Golgi membranes in vitro (Fig. 3), we specifically tested whether the 1–122 versus the 1–172 chimeras (7 and 9 in Table 1) displayed distinct subcellular localization. The micrographs reveal that both constructs have similar drebrin-like localization and colocalize with cell-surface actin (Supplemental Fig. 3). Thus, consistent with Fig. 6, the presence of the drebrin charged/helical domain appears to dominate with regard to subcellular localization.

The dominant contribution of the drebrin charged/helical domain hindered our ability to characterize independent contributions from the ADFH/linker domains. Our in vitro binding experiments suggested that the mAbp1 ADFH/linker domain was distinct in that it bound to a cytochalasin D-dependent actin pool (Fig. 4B). Hence, we examined the effects of cytochalasin D on mAbp1 and drebrin distribution in whole cells. After cytochalasin D treatment, the microfilaments were largely disorganized and often appeared as phalloidin-stained large aggregates (Fig. 7). Drebrin was found to colocalize with F-actin both in the presence and absence of cytochalasin D, presumably via the charged/helical domain (Fig. 7A). By contrast, the colocalization of mAbp1 with F-actin was enhanced upon cytochalasin D addition (Fig. 7B). The result is consistent with our findings in vitro that the ADFH domain of mAbp1 can bind to a specific type of actin structure that is apparently stabilized by the plus-end binding toxin cytochalasin D.

Together, our results show that the two conserved actin binding domains of drebrin and mAbp1, although conserved, each form distinct interactions with the actin cytoskeleton. We propose that the properties of these motifs direct the subcellular localization and membrane binding properties of these two proteins.

**DISCUSSION**

Previous characterization of the related actin-binding proteins drebrin and mAbp1 revealed both shared and distinct properties. Both proteins bind F-actin and bind to Golgi membranes in an ARF1-dependent manner. However, the proteins respond differently to disrupting actin dynamics on Golgi membranes and display distinct subcellular localization. We initially anticipated that the shared properties might be conferred by the two conserved actin binding domains, whereas their distinct properties might be conferred by non-conserved C-terminal motifs. Instead, the results presented here show that the ADFH and charged/helical actin binding domains are sufficient and necessary for the localization and binding properties of drebrin and mAbp1.

Characterization of the ADFH domains (4 and 13 in Table 1) showed that the ability to bind F-actin is insufficient for correct subcellular localization and regulated binding to Golgi membranes. The charged/helical actin binding domain was required together with ADFH domain for correct binding and localization. Our results further revealed that a linker region between the two previously defined actin binding domains (mAbp1 residues 123–171) conferred the ability of mAbp1 to associate with the cytochalasin D-resistant actin pool on Golgi membranes. This linker motif also affected the sensitivity of chimeric proteins to disrupting coatomer/Cdc42-mediated signaling with a peptide corresponding to the C-terminal coatomer binding domain of p23. A comparison of the amino acid sequence between drebrin and mAbp1 reveals that the linker region is poorly conserved, with only 2 identities between residues 122 and 156. Together, our data reveal that the divergent sequence between the two actin binding domains plays a role in the ability of these proteins to associate with distinct actin structures on the Golgi membranes.

In our study we have identified an additional sequence motif within the charged/helical region (drebrin residues 173–227) that influences the ability of drebrin and mAbp1 to associate with the cell surface and induce filopodia formation. Surprisingly, the sequence of this charged region between residues 173 and 227 is conserved (36 of 55 identities or conservative changes) despite the fact that this motif had dramatic effects on the subcellular localization of these proteins. A previous study identified a region within drebrin that contributes to actin binding, subcellular localization, and the induction of filopodia formation (24). The authors of this study concluded that additional motifs must also contribute to the subcellular localization of drebrin. We suggest that the region between amino acids 173 and 227 corresponds to the motif predicted in the previous study.

Perhaps our most remarkable finding is that the conserved ADFH and charged/helical actin binding domains are not equivalent. The ADFH/linker domain of mAbp1 confers binding to a cytochalasin D-dependent actin pool; however, the actin binding domains of drebrin do not have this property. Furthermore, the charged/helical domain of drebrin directs binding to cell-surface actin and increases the number of filopodia. The homologous domain from mAbp1 directs localization to internal structures.
A diverse set of actin-based structures is required within cells. The actin cytoskeleton facilitates the formation of complex cell morphological features such as microvilli and neuronal dendrites. Myosin-dependent motility and contractility requires motor protein movement along actin microfilaments. Dynamic actin rearrangements within cell-surface structures like lamellipodia and filopodia are essential for cell motility. Polymerization of actin into comet tails propels organelles and vesicles within cells as well as pathogens such as *Listeria*. Although the basic building blocks for these actin-based structures are microfilaments, they are able to interact with distinct sets of F-actin-binding proteins to create this diverse array of actin structures and functions. It is not clear how actin microfilaments recruit a distinct set of actin-binding proteins to allow correct localization and the formation of a specific structure.

When taken together, our results suggest that actin-binding proteins...
are not targeted solely through a general binding interaction with actin microfilaments but, instead, through a more complex mechanism that involves multiple interactions with distinct types of actin-based complexes.

Acknowledgment—We thank Jilong Chen for helpful discussions.

REFERENCES

1. Lee, M. C., Miller, E. A., Goldberg, J., Orci, L., and Schekman, R. (2004) Annu. Rev. Cell Dev. Biol. 20, 87–123
2. Stamnes, M. (2002) Curr. Opin. Cell Biol. 14, 428–433
3. Chen, J. L., Lacomis, L., Erdjument-Bromage, H., Tempst, P., and Stamnes, M. (2004) FEBS Lett. 566, 281–286
4. Duran, J. M., Valderrama, F., Castel, S., Magdalena, J., Tomas, M., Hooya, H., Renaud-Piqueras, J., Mallotra, V., and Egea, G. (2003) Mol. Biol. Cell 14, 445–459
5. Fucini, R. V., Chen, J. L., Sharma, C., Kessels, M. M., and Stamnes, M. (2002) Mol. Biol. Cell 13, 621–631
6. Fucini, R. V., Navarrete, A., Vadakkan, C., Lacomis, L., Erdjument-Bromage, H., Tempst, P., and Stamnes, M. (2000) J. Biol. Chem. 275, 18824–18829
7. Valderrama, F., Luna, A., Babia, T., Martinez-Menarguez, J. A., Ballesta, J., Barth, H., Chaponnier, C., Renaud-Piqueras, J., and Egea, G. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1560–1565
8. Dubois, T., Paleotti, O., Mironov, A. A., Fraisier, V., Stradal, T. E., De Matteis, M. A., Franco, M., and Chavrier, P. (2005) Nat. Cell Biol. 7, 353–364
9. Godi, A., Santone, I., Pertile, P., Devrajaj, P., Stabach, P. R., Morrow, J. S., Di Tullio, G., Polishchuk, R., Petrucci, T. C., Luini, A., and De Matteis, M. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8607–8612
10. Luna, A., Matas, O. B., Martinez-Menarguez, J. A., Mato, E., Duran, J. M., Ballesta, J., Way, M., and Egea, G. (2002) Mol. Biol. Cell 13, 866–879
11. Matas, O. B., Martinez-Menarguez, J., and Egea, G. (2004) Traffic 5, 838–846
12. Donaldson, J. G. (2002) Methods Mol. Biol. 189, 191–198
13. Hirono, M., and Exton, J. H. (2005) J. Cell. Physiol. 202, 608–622
14. Nie, Z., Hirsch, D. S., and Randazzo, P. A. (2003) Curr. Opin. Cell Biol. 15, 396–404
15. Wu, W. J., Erickson, J. W., Lin, R., and Cerione, R. A. (2000) Nature 405, 800–804
16. Chen, J. L., Fucini, R. V., Lacomis, L., Erdjument-Bromage, H., Tempst, P., and Stamnes, M. (2005) J. Cell Biol. 169, 383–389
17. Cao, H., Weller, S., Ortz, J. D., Chen, J., Huang, B., Chen, J. L., Stamnes, M., and McNiven, M. A. (2005) Nat. Cell Biol. 7, 483–492
18. Kaksonen, M., Toret, C. P., and Drubin, D. G. (2005) Cell 123, 305–320
19. Newpher, T. M., Smith, R. P., Lemmon, V., and Lemmon, S. K. (2005) Dev. Cell 9, 87–98
20. Wesp, A., Hicke, L., Palecek, J., Lombardi, R., Aust, T., Munn, A. L., and Riezman, H. (1997) Mol. Biol. Cell 8, 2291–2306
21. Kessels, M. M., Engqvist-Goldstein, A. E., Drubin, D. G., and Qualmann, B. (2001) J. Cell Biol. 153, 351–366
22. Butkevich, E., Hulsmann, S., Wenzel, D., Shirao, T., Duden, R., and Majoul, I. (2004) Curr. Biol. 14, 650–658
23. Inoue, H. K., and Shirao, T. (1997) J. Electron Microsc. 46, 497–502
24. Hayashi, K., Ishikawa, R., Kawai-Hirai, R., Takagi, T., Taketomi, A., and Shirao, T. (1999) Exp. Cell Res. 253, 673–680
25. Takahashi, H., Sekino, Y., Tanaka, S., Mizui, T., Kishi, S., and Shirao, T. (2003) J. Neurosci. 23, 6586–6595
26. Quintero-Monzon, O., Rodal, A. A., Strokopytov, B., Almo, S. C., and Goode, B. L. (2005) Mol. Biol. Cell 16, 3128–3139
27. Percival, J. M., Hughes, J. A. I., Brown, D. L., Schezov, G., Heimann, K., Vrhovski, B., Bryce, N., Stow, J. L., and Gunning, P. W. (2004) Mol. Biol. Cell 15, 268–280
28. Mallotra, V., Serafini, T., Orci, L., Shepherd, J. C., and Rothman, J. E. (1989) Cell 58, 329–336
29. Ahluwalia, J. P., Topp, J. D., Weirather, K., Zimmerman, M., and Stamnes, M. (2001) J. Biol. Chem. 276, 34158–34165
30. Kessels, M. M., Engqvist-Goldstein, A. E., and Drubin, D. G. (2000) Mol. Biol. Cell 11, 393–412