The Carboxyl-terminal Region of EBP50 Binds to a Site in the Amino-terminal Domain of Ezrin That Is Masked in the Dormant Molecule*

David Reczek and Anthony Bretscher‡
From the Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

EBP50 (ezrin-radixin-moesin-binding phosphoprotein 50) was recently identified by affinity chromatography on the immobilized NH2-terminal domain of ezrin. Here we map and characterize the regions in EBP50 and ezrin necessary for this association. Using blot overlays and in solution binding assays, the COOH-terminal 30 residues of EBP50 were found to be sufficient for an association with residues 1–286 of ezrin. EBP50 did not bind to full-length (1–585) ezrin, indicating that the EBP50 binding site is masked in the full-length molecule. Ezrin contains two complementary self-association domains known as N- and C-ERMADs (ezrin-radixin-moesin-association domains), encompassing residues 1–296 and 479–585, respectively. An ezrin 1–583 construct lacking the two terminal residues necessary for this association was found to have an unmasked EBP50 binding site. Moreover, binding of EBP50 and the C-ERMAD to ezrin residues 1–296 was found to be mutually exclusive, with the C-ERMAD having a higher affinity. These results suggest that in full-length ezrin, the binding site for EBP50 is masked through an intramolecular N/C-ERMAD association. Based on these and additional results, we propose a model whereby dormant ezrin can be activated to bind EBP50 on its NH2-terminal end and F-actin on its COOH-terminal end. Since EBP50 is proposed to bind membrane proteins through its PDZ domains, this provides a molecular description of the regulated linkage of microfilaments to membranes in cell surface microvilli.

Polarized epithelial cells assemble distinct apical and basolateral domains for the transport of small molecules. The apical domain is generally characterized by the presence of abundant microvilli in which reside specific membrane-bound transport proteins. Recent studies at understanding the mechanism of microvillus formation have focused on ezrin, a member of the ezrin-radixin-moesin (ERM)1 protein family that are about 580 residues in length, about 60% identity to ERM proteins over this same region (8, 9). The ERM proteins, which are about 580 residues in length, have a ~300 residue NH2-terminal domain that can interact with the cytoplasmic domain of the transmembrane protein CD44 (10), the PDZ domain-containing ERM-binding phosphoprotein 50 (EBP50) (11, 12), or the Rho-GDI dissociation inhibitor (Rho-GDI) (13). Following this domain is a region predicted to be largely α-helical. An F-actin binding site has been mapped to the 34 COOH-terminal residues (14, 15), although recent studies suggest that association with actin might be more complex (16).

In addition to these potential interactions, the ERM proteins have the ability to form homo- and heterotypic associations between family members (17). The first 296 residues of each member defines a domain that can form a high affinity association with the 107-residue COOH-terminal region of any ERM protein; these have been called NH2- and COOH-ERM association domains (N- and C-ERMADs) (18). These self-association domains are not constitutively available for spontaneous oligomerization in vivo, as the bulk of ezrin exists in the cytoplasm in a monomeric form (19, 20). Studies with native monomeric ezrin have revealed that the C-ERMAD, as well as the COOH-terminal F-actin binding site, is conformationally masked (18). Regulated self-association seems to be physiologically relevant as dimers and other oligomers are the predominant forms of ezrin in isolated placental microvilli, and dimer formation can be induced in cultured cells by the activation of signaling pathways in a time course that parallels microvillus assembly (21). These combined results led us to propose that the bulk of the ezrin in cells exists in a dormant monomeric conformation in which sites for self-association and association with other proteins are masked. Ezrin activation is proposed to lead to the exposure of sites for both oligomerization and interaction with membrane and cytoskeletal components (21, 1).

We recently described human EBP50, a 358-residue protein that was identified by affinity chromatography on resins containing the NH2-terminal domains of ezrin or moesin (11). Analysis of the EBP50 sequence revealed that it contains two PDZ domains followed by a 120-residue COOH-terminal tail. Localization of EBP50 in tissues and cultured cells shows that it colocalizes with ezrin in apical microvilli. The existence of complexes of ezrin and EBP50 in extracts of isolated microvilli indicates that the binding of EBP50 to ezrin is of physiological significance. The probable rabbit homologue of human EBP50, the Na+ /H+ exchanger regulatory factor, has been identified as a protein necessary for the cAMP-dependent protein kinase A regulation of the renal Na+/H+ exchanger (22, 23). A closely related protein, the NHE3 kinase A regulatory protein...
(E3KARP), was identified in a two-hybrid interaction screen using the cytoplasmic domain of the renal Na+/H+ exchanger NHE3 isoform as bait (24). Thus, a likely scenario places EBP50 as an adaptor protein between NHE3 and ezrin at least in the renal brush border. Very recently, EBP50 was identified in another two-hybrid screen, this time using the NH2-terminal domain of merlin as bait (12).

In this paper, we identify and characterize the regions in ezrin necessary for interaction with EBP50 and the regions in EBP50 necessary for an interaction with ezrin. Since the NH2-terminal domain of ezrin can bind both EBP50 and the ezrin C-ERMAD, we also examine the relationship between these binding sites. Our results show that the binding of EBP50 and the C-ERMAD to the ezrin NH2-terminal domain are mutually exclusive and hierarchical and that both binding sites are masked in the full-length molecule. The masking of the EBP50 binding site in dormant ezrin provides further biochemical evidence that ezrin has to be activated for its membrane-cytoskeletal linking function. These findings allow us to propose a model of molecular linkages that lead to the attachment of microfilaments to the plasma membrane.

**EXPERIMENTAL PROCEDURES**

Production and Purification of Recombinant Proteins—The design and production of the glutathione S-transferase (GST)-ezrin deletion series (18) and of the EBP50 and Ez-(1–296) constructs have been described (11). The full-length Ez-(1–585) and the Ez-(479–585) (C-ERMAD) constructs were created by polymerase chain reaction amplification of the respective cDNA sequences from clone F6 (4) using primers that generated EcoRI and HindIII sites at their ends. These products were then subcloned into the expression vector pQE16 (QIAGEN Inc., Chatsworth, CA). The series of GST-EBP50 proteins containing NH2- and COOH-terminal deletions was created by a combination of polymerase chain reaction amplification and subcloning of sequences from the human EBP50 cDNA (GenBankTM accession number AF015926). The polymerase chain reaction products that had been generated with unique restriction sites at their ends and the fragments to be subcloned were directionally ligated into the polylinkers of the appropriate pGEX series fusion vectors (Amersham Pharmacia Biotech). All recombinant sequences were determined to be free of polymerase chain reaction errors by nucleotide sequence analysis. Restriction enzymes and other reagents for molecular biology were purchased from Life Technologies, Inc.

The expression of the GST-ezrin series constructs (18) and of the Ez-(1–296) construct (11) has been described. GST-EBP50 deletion series constructs were transformed into the Escherichia coli strain DH5α and expressed like the GST-ezrin constructs. For the expression of EBP50 and Ez-(479–585), the plasmids encoding these constructs were transformed into the E. coli strain JM109. Truncated murine dihydrofolate reductase, encoded on the QIAGEN pQE16 vector, was also expressed in JM109. Saturated overnight cultures were inoculated at 1:20 dilution in LB medium containing 100 μg/ml ampicillin and grown for 90 min at 37 °C. Isopropyl-β-D-thiogalactopyranoside was added to 2 mm, and cells were grown for an additional 180 min. Cells were harvested by centrifugation at 8000 × g for 15 min. Gel samples of total soluble bacterial lysates were prepared by resuspending the soluble lysates in 1 volume of SDS sample buffer (25) and boiling for 2 min.

The purification of the bacterially expressed Ez-(1–296) was performed in essentially the same manner (sequential KH2PO4 linear gradient. Homogenous ezrin eluted at 300 mM NaCl, the beads were put through a second round of incubation and washing, and bound proteins were extracted. In the case where EBP50- or C-ERMAD-containing lysate was added in increasing amounts, reticulation volumes were normalized with Tris-buffered saline.

**SDS-Polyacrylamide Gel Electrophoresis, Blot Overlays, and Immuno- blots**—SDS-polyacrylamide gel electrophoresis was performed as described previously (25), and protein samples were analyzed on 10, 11, or 13% gels. In some cases, gels were stained with Coomassie Brilliant Blue R-250. For blots, proteins were transferred from gels to polyvinylidene membranes (Millipore Corp., Bedford, MA) using a semidry electrophoretter (Integrated Separation Systems, Hyde Park, MA). All blots were developed using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech). In some cases, blots were stained with Coomassie Brilliant Blue R-250 after development to assess the efficiency of protein transfer.

Biotinylated Ez-(1–296) and EBP50 probes were prepared, and blot overlays were performed as described (17). The biotinylated probes were oriented in control experiments. Peroxidase-conjugated avidin (Sigma) was used to detect biotin-labeled proteins.

Immunoblots were blocked with 10% nonfat dry milk, then probed with a 1:10000 dilution of GST antiserum (Amersham) in 1% milk, followed by 0.1 μg/ml peroxidase-conjugated goat anti-rabbit IgG in 1% milk.

**RESULTS**

Mapping the Ezrin-EBP50 Interaction Sites—The NH2-terminal domain of ezrin (Ez-(1–296)) binds to EBP50 with high affinity in solution and by blot overlay (11). To define the region in EBP50 that interacts with Ez-(1–296), a series of recombinant GST-EBP50 fusion proteins containing intact EBP50 or NH2- and COOH-terminal deletions, was generated and expressed in bacteria (Fig. 1). Analysis of lysates of induced bacteria show that the major induced polypeptides correspond to the GST fusion proteins (Fig. 1, upper and middle panels). Some of the GST-EBP50 fusion proteins exhibited an anachronously slow migration, like that seen for native EBP50 (11). Plots of Rf versus log molecular weight indicated that the regions of EBP50 responsible for the majority of this retardation lie within residues 97–138 and 242–320. To determine the region in EBP50 necessary for association with Ez-(1–296), a blot overlay assay was employed in which biotinylated recombinant Ez-(1–296) was used as a probe on the GST-EBP50 fusion protein series (Fig. 1, bottom panel). The Ez-(1–296) probe bound only to those EBP50 constructs that contained an intact COOH terminus. A 61-residue COOH-terminal truncation was not recognized (Fig. 1, lane B), whereas all constructs that contained the COOH-terminal 30 residues (329–358) of EBP50 bound Ez-(1–296).

Although we have previously found that Ez-(1–296) binds to EBP50 in vitro, we wished to investigate whether additional regions in ezrin bind to EBP50 and to further refine the minimal region necessary for this interaction. Use was made of the GST-ezrin fusion constructs described previously (18) that contain NH2- and COOH-terminal deletions of ezrin fused to GST (Fig. 2, top panel), Coomassie stain; middle panel, GST immuno- blot). Since biotinylated EBP50 will bind to Ez-(1–296) in blot overlays (11), this technique was used to examine the regions in ezrin necessary for interaction with EBP50. Only those constructs that contained the first 286 residues of ezrin bound the probe, demonstrating that these residues alone were sufficient for the binding of EBP50 (Fig. 2, bottom panel).

Remarkably, EBP50 failed to bind to the full-length ezrin fusion protein, whereas it did bind to a construct lacking just two
COOH-terminal residues (1–583) (Fig. 2, lanes A and B, respectively).

These results, summarized in Fig. 3, indicate that the COOH-terminal 30 residues of EBP50 interact specifically with the NH2-terminal 286 residues of ezrin and that this interaction can, at least by blot overlay, be inhibited by a COOH-terminal region of ezrin.

The Binding Sites for EBP50 and the C-ERMAD Are Masked in the Native Ezrin—The N-ERMAD (Ez-(1–296)) and C-ERMAD (Ez-(479–585)) of ezrin bind each other with high affinity in vitro. In native full-length ezrin, the C-ERMAD is masked and therefore unable to bind a free ectopic N-ERMAD (18). To explore whether or not the binding site for EBP50 in Ez-(1–296) is accessible in native ezrin, we compared the ability of full-length ezrin and Ez-(1–296) to bind recombinant EBP50. Additionally, since it has not been determined whether the N-ERMAD is accessible in full-length ezrin, we also compared the abilities of full-length ezrin and Ez-(1–296) to bind recombinant C-ERMAD.

Untagged, recombinant versions of full-length ezrin and Ez-(1–296) were expressed as soluble proteins in bacteria and purified to homogeneity (Fig. 4A). The purified proteins were then coupled covalently to beads to make affinity resins. Total soluble bacterial lysates expressing either a portion of the murine dihydrofolate reductase protein as a nonspecific control or untagged EBP50 or untagged C-ERMAD were prepared (Fig. 4B) and incubated with each set of beads. After extensive washing in high salt buffer, the bound proteins were eluted by boiling the beads in SDS sample buffer and analyzed by gel electrophoresis (Fig. 4C). Neither EBP50 nor the C-ERMAD bound to full-length ezrin (Fig. 4C, lanes 3 and 5, respectively), whereas both bound to Ez-(1–296) (Fig. 4C, lanes 4 and 6, respectively). No proteins from the control lysate bound to either set of beads (Fig. 4, lanes 1 and 2). Thus, the binding sites for EBP50 and an ectopic C-ERMAD are masked in native ezrin.

How might this masking occur? Given that the C-ERMAD itself is masked in native ezrin (18) and having demonstrated that the N-ERMAD is inaccessible to ectopic C-ERMAD, we considered the possibility that masking in the full-length protein was due to an intramolecular N/C-ERMAD association. Since removal of the last two residues from full-length ezrin abolishes the activity of its endogenous C-ERMAD (18), we generated and purified Ez-(1–583) lacking the last two COOH-terminal residues. If masking is normally dependent on an intramolecular association between N- and C-ERMADs, then binding sites that are normally cryptic in the full-length molecule should now be accessible in this truncated form. To examine this possibility, saturating amounts of lysates of Ez-(1–296), Ez-(1–583), and full-length ezrin (Fig. 4D) were each incubated with beads containing immobilized GST-EBP50 COOH-terminal fusion protein (residues 320–358). After extensive washing, bound proteins were eluted and analyzed by gel electrophoresis. Both Ez-(1–296) and Ez-(1–583) bound
tightly to the EBP50 beads (Fig. 4E, lanes 2 and 3, respectively), whereas the binding of full-length ezrin was negligible (lane 4). No proteins from a control lysate bound to the beads (lane 1), and no specific binding of any proteins from the lysates bound to GST control beads (data not shown). These data suggest that the masking of the EBP50 binding site and of the N- and C-ERMDs in native ezrin is a consequence of an intramolecular N/C-ERMAD association in full-length native monomeric ezrin.

**EBP50 and the C-ERMAD Have Mutually Exclusive Binding Sites on Ez-(1–296)—**The region in ezrin required for binding EBP50 lies within residues 1–286 (Figs. 2 and 3), whereas the region necessary for binding the C-ERMAD lies in residues 1–296, with 1–286 being insufficient for this interaction (18). We therefore wished to determine whether Ez-(1–296) was able to bind EBP50 and the C-ERMAD simultaneously or if their binding was mutually exclusive.

Our strategy was to saturate immobilized Ez-(1–296) with EBP50 and then to examine whether EBP50 could be displaced by the subsequent addition of C-ERMAD. The quantity of EBP50-containing bacterial lysate needed to saturate all the Ez-(1–296) sites was determined by adding increasing amounts of lysate to replicate sets of beads. The beads were washed extensively, and bound EBP50 was eluted and analyzed by gel electrophoresis (Fig. 5A). Increasing amounts of lysate resulted in larger amounts of bound EBP50 (lanes 1–5) until a saturation point was reached where the addition of more lysate did not result in any further increase (lanes 6–7). This amount of EBP50-containing lysate was used to saturate replicate sets of Ez-(1–296) beads that were then challenged with increasing amounts of C-ERMAD-containing lysate (Fig. 5B). As the amount of lysate increased, bound C-ERMAD increased and bound EBP50 decreased (lanes 1–5) until a point at which the addition of more C-ERMAD lysate did not result in any further increase in bound C-ERMAD (lanes 6–7). These results indicate that the C-ERMAD can displace the bulk of EBP50 from Ez-(1–296).

Why does some EBP50 remain bound, even at saturating amounts of C-ERMAD? Repeated challenging with additional saturating amounts of C-ERMAD did not displace any more of the residual EBP50 (data not shown), indicating that this population is bound in a C-ERMAD-insensitive fashion. Since the binding of the C-ERMAD requires ezrin residues 1–296, whereas EBP50 requires only 1–286, it is likely that a portion of the immobilized Ez-(1–296) molecules are coupled in such a way as to allow the binding of EBP50 but to preclude the binding of the C-ERMAD.

To explore the hierarchy of C-ERMAD and EBP50 binding further, the effect of changing the order of ligand addition was also examined. Lysates from bacteria expressing either the

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**Fig. 3. Summary of EBP50-ezrin association site mapping.** Schematic representations of ezrin and EBP50 are shown as boxes, with residues 1–296 of ezrin and the PDZ domains of EBP50 shaded. Lines denote the relative coding regions expressed for each construct.

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**Fig. 4. The binding sites for EBP50 and the C-ERMAD are masked in native ezrin.** A, Coomassie Blue-stained 13.3% gel of the purified ezrin (lane 1) and Ez-(1–296) (lane 2) that were covalently coupled to Sepharose beads and used in binding assays. B, Coomassie Blue-stained 13.3% gel of the total soluble induced bacterial lysates used in binding assays: lane 1, control dihydrofolate reductase; lane 2, EBP50; lane 3, ezrin C-ERMAD. Arrowheads denote the locations of the recombinant proteins in the lysates. C, affinity binding assay comparing the accessibility of EBP50 and C-ERMAD binding sites in full-length native ezrin versus Ez-(1–296). Ezrin beads (lanes 1, 3, and 5) or Ez-(1–296) beads (lanes 2, 4, and 6) were incubated with soluble induced bacterial lysates (lanes 1 and 2, control lysate; lanes 3 and 4, EBP50 lysate; lanes 5 and 6, C-ERMAD lysate), washed extensively in high salt buffer, then extracted by boiling in SDS sample buffer, and bound proteins were resolved on an 13.3% gel. D, Coomassie Blue-stained 11% gel of the total soluble induced bacterial lysates used in the GST-EBP50 (320–358) bead binding assay: lane 1, Ez-(1–296); lane 2, Ez-(1–585); lane 3, Ez-(1–585) (full-length). Arrowheads denote the locations of the recombinant proteins in the lysates. E, affinity binding assay examining the accessibility of the EBP50 binding site in COOH-terminally truncated ezrin. Glutathione beads, to which the GST-EBP50 (320–358) fusion protein was immobilized, were incubated with soluble induced bacterial lysates (lane 1, control dihydrofolate reductase; lane 2, Ez-(1–296); lane 3, ezrin 1–583; lane 4, ezrin 1–585), then washed extensively in high salt buffer, extracted from as above, and resolved on an 11% gel. The migration position of the fusion protein (FP) is indicated. The mobility of molecular mass standards in kDa is indicated at left. DF, dye front.
dihydrofolate reductase fragment, untagged EBP50, or the untagged ezrin C-ERMAD were each added in sufficient quantities to replicate sets of beads to saturate all the sites on the coupled Ez-(1–296). The beads were washed extensively in high salt buffer, one set eluted, and the bound proteins were analyzed (Fig. 5C). As expected, EBP50 and the C-ERMAD were specifically and efficiently retained (lanes 1–3). The second set of Ez-(1–296) beads, to which EBP50 or the C-ERMAD were already bound, was challenged with bacterial lysates containing the expressed dihydrofolate reductase fragment, washed extensively, and eluted (lanes 4 and 5). There was no diminution of the amount of EBP50 or C-ERMAD retained by treatment with this nonspecific control extract. In the third set of beads, each was challenged with lysates containing the alternate ligand. When beads with bound C-ERMAD were challenged with EBP50, there was no diminution in the amount of bound C-ERMAD, but a small amount of EBP50 was also retained (lane 7). However, when beads with bound EBP50 were challenged with C-ERMAD, the bulk of the EBP50 was displaced (lane 6), and the beads bound an amount of C-ERMAD equivalent to the saturated controls (lanes 3 and 5).

These results indicate that EBP50 cannot readily displace bound C-ERMAD from immobilized Ez-(1–296), whereas the C-ERMAD can displace the bulk of the EBP50, indicating that the binding of the two proteins to Ez-(1–296) is mutually exclusive. Furthermore, they show that the C-ERMAD binds Ez-(1–296) with greater affinity than EBP50.

DISCUSSION

In this study we have investigated the sites of interaction between EBP50 and ezrin. The results show that the COOH-terminal 30 residues of EBP50 are sufficient for an association with ezrin and that the EBP50 binding site in ezrin lies within the first 286 residues where it is masked in the native molecule. We have also shown that the binding of EBP50 to Ez-(1–296) can be inhibited by the C-ERMAD (Ez-(479–585)). These results have interesting implications for the modes of action of both EBP50 and ezrin.

Human EBP50 is 55% identical to human E3KARP, a 337-residue protein with two PDZ domains that interacts directly with the cytoplasmic domain of the Na+/H+ exchanger NHE3 isoform (24). The COOH-terminal 30 residues of EBP50 that bind ezrin are especially well conserved in E3KARP (Fig. 6), suggesting that E3KARP might also bind Ez-(1–296). This proposal is supported by preliminary experiments in which a GST-E3KARP fusion protein was found to bind Ez-(1–296) (or moesin residues 1–286) with high affinity. In addition, ezrin and band 4.1 have been reported to bind to hDlg (26, 27), the human homolog of the Drosophila discs large protein. Inspection of the region of hDlg believed to be responsible for this association revealed limited sequence identity to the COOH-terminal regions of EBP50 and E3KARP (Fig. 6). It is therefore possible that the nature of the interaction between ezrin and EBP50 is similar to that between ezrin or band 4.1 and hDlg.

So far there is no evidence to indicate that the binding of EBP50 to Ez-(1–296) is regulated at the level of EBP50, since full-length and truncated forms of EBP50 containing the COOH-terminal 30 residues bind Ez-(1–296), and immobilized Ez-(1–296) binds to different phosphorylated forms of EBP50 present in tissue extracts (11). By contrast, the binding site for EBP50 is masked in full-length ezrin. When a series of bacterially expressed GST-ezrin deletion constructs were tested for their ability to bind an EBP50 probe in a blot overlay assay, all constructs containing ezrin residues 1–286 bind EBP50, with the remarkable exception of the full-length (1–585) construct. Removal of two COOH-terminal residues from ezrin restored the ability of EBP50 to bind it in the blot overlay assay (Fig. 2). The same phenomenon was seen when the binding of EBP50 to Ez-(1–296) was assayed by a solution assay (Fig. 4E). Since removal of these two residues destroys the ability of the C-ERMAD to bind the N-ERMAD (18), the most likely interpretation is that the C-ERMAD inhibits the binding of EBP50 to Ez-(1–296). We therefore suggest that in full-length ezrin, the C-ERMAD forms an intramolecular association with the N-ERMAD, thereby masking the potential EBP50 binding site in Ez-(1–296). Such a scenario would imply that EBP50 and the C-ERMAD cannot bind Ez-(1–296) simultaneously and that bound C-ERMAD cannot be displaced by soluble EBP50. These predictions are supported by our solution binding results (Figs. 4 and 5).

D. Roczek and A. Bretscher, unpublished data.
What is the relationship between the binding sites on Ez-(1–296) for EBP50 and the C-ERMAD (Ez-(479–585))? Several observations suggest that the two binding sites are not identical. First, the C-ERMAD requires ezrin residues 1–296 for full activity (18), whereas EBP50 binds to constructs containing only residues 1–286. The additional 10 residues needed for C-ERMAD binding are highly basic (pI = 10.98) and the C-ERMAD is more acidic (pI = 5.45) than the 30 residues in EBP50 sufficient for binding ezrin (pI = 10.56), suggesting that charge interactions in this region may be important for C-ERMAD binding. Second, immobilized Ez-(1–296) has sites that are accessible to EBP50 but not to the C-ERMAD. Third, there is no obvious sequence homology between the C-ERMAD and the COOH-terminal tail region of EBP50 that binds to Ez-(1–296). These results suggest that the binding sites are either distinct or overlap in such a way that binding of the C-ERMAD reduces the affinity of EBP50 for Ez-(1–296). If the sites are distinct, binding of the C-ERMAD must induce a conformational change in Ez-(1–296) that reduces its affinity for EBP50. Future structural analyses will be needed to distinguish unambiguously between these possibilities.

We have so far considered the interaction of Ez-(1–296) with EBP50 and the C-ERMAD. However, this same domain has also been reported to bind to both Rho-GDI (13) and the cytoplasmic tail of the transmembrane protein CD44 (10). Like EBP50, Rho-GDI can bind to a domain consisting of residues 1–296, and this binding can be abrogated by subsequent addition of a C-ERMAD (13). Since the C-ERMAD, EBP50, Rho-GDI, and the cytoplasmic tail of CD44 do not share any obvious sequence homology, it will be important to determine how many binding sites exist on Ez-(1–296) and, if mutually exclusive, what their binding hierarchy is.

How do these results relate to the current ideas of ezrin function? Previous studies have demonstrated that the C-ERMAD of ezrin, which contains the COOH-terminal F-actin binding site, is masked in the dormant full-length molecule (18). Moreover, the site in Ez-(1–296) for association with CD44 is partially masked in full-length ezrin (10) as is the site for association with Rho-GDI (13). In addition, we now show that the N-ERMAD is masked in the dormant full-length molecule. The finding that dormant ezrin does not bind EBP50, whereas EBP50/ezrin complexes can be isolated from purified placental membranes, suggests that for ezrin or whether the EBP50 binding site is masked in full-length merlin. Resolution of these points will determine how EBP50 is partitioned between ERM members and merlin and how this is altered by the stimulation of signal transduction pathways that activate ERM members. Thus, it appears that we are just beginning to glimpse the intricate and regulated nature of the associations in which the ezrin family of proteins can participate. Further studies will have to examine how ezrin selects among its many suitors, what the physiological role of each association is, and why a single protein participates in so many regulated associations.

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