Complex structure of Crumbs cytoplasmic tail with Moesin FERM domain reveals a novel FERM-binding mode

by

Li Youjun

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June 14, 2012
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Li Youjun

This is to certify that I have examined the above MPhil thesis and have found that it is complete and satisfactory in all respects, and that any and all revisions required by the thesis examination committee have been made.

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June 14, 2012

III
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Complex structure of Crumbs cytoplasmic tail with Moesin FERM domain reveals a novel FERM-binding mode

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Abstract

The apical transmembrane protein Crumbs is a determinant of apical-basal cell polarity. The short cytoplasmic domain of Crumbs, containing two highly conserved motifs, a PSD95/Discs-large/ZO1 (PDZ) binding motif (PBM) and a 4.1/ezrin/radixin/moesin (FERM)-binding motif (FBM), plays an essential role for Crumbs’ function in definition of the apical boundary during development. It has been widely accepted that Crumbs forms a critical polarity complex with a membrane-associated guanylate kinase (MAGUK) family protein Pals1 and a PDZ-containing protein PATJ via the PBM/Pals1 interaction. However, the roles of FBM in Crumbs are poorly understood. Recently, Médina et al reported that Crumbs interacts with a FERM domain-containing protein Moesin via its FBM. As Moesin connects to cytoskeleton, the Crumbs/Moesin interaction provides a potential mechanism to stabilization of the apical domain.

Here, we biochemically and structurally characterized the interaction between the cytoplasmic domain of Crumbs and the FERM domain of Moesin. We confirmed that the FBM is required for the Crumbs/Moesin interaction. Surprisingly, we further identified that the PBM also contributes to the interaction. We determined the crystal structure of the Crumbs/Moesin complex at 1.5 Å resolution. Consistent with our biochemical data, the complex structure shows that both the FBM and PBM are involved in the binding of Crumbs to the FERM domain of Moesin. Together, our findings indicate that the two motifs of Crumbs not only act as binding sites but also regulate the interactions between Crumbs and their binding partners. This finding provides a direct linkage between the establishment of the apical-basal polarity and the stabilization of the apical domain together. Additionally, the PBM in the Crumbs/Moesin complex occupies the InsP₃ binding site in the Moesin FERM domain, suggesting that Crumbs may compete the binding of Moesin with plasma membrane.
Chapter 1

Introduction

Polarization of epithelial cell is a complicated but ordered process, involved in a series of events including cell-cell adhesion, assembly of cell cortex, cytoskeleton reorganization and transportation of vesicles to specified domains. The maintenance of epithelial cell polarity depends on cell adhesion complex and cell polarity complex. Adhesion junctions and tight junctions contribute to cell adhesion complex in simply epithelial, despite of the minor difference of tight junctions in neuro-epithelial cells and stratified epithelial[1, 2]. The junction formation is actin-based and plays a leading role in the formation of apical-basal cell polarity. Cell polarity complex including Crumbs-Pals1-PATJ complex, Par3-Par6-aPKC complex and Scribble-Dlg-Lgl complex have been identified in the last 20 years, which play an essential role in promoting the establishment of the apical-basal border and stabilizing the apical and basal regions[3-8].

Cell polarity endues cells to sense and interpret different signals from neighbourings and microenvironment, in which transmembrane proteins and membrane receptors take up major if not all responsibility. Crumbs is a transmembrane protein contains a large extracellular domain, composed of 29 epidermal growth factor (EGF)-like repeats and four laminin-A globular domain like repeats, and a very short yet versatile cytoplasmic region of about 38 amino acids[9-12]. The small, 38-amino-acid cytoplasmic region covers the majority of Crumbs functions in apical basal cell polarity, as overexpression of full length or just cytoplasmic region of Crumbs has the similar rescue phenotype in crb mutant epithelial cells[13]. Three highly conversed sites are integrated in this short cytoplasmic region including a potential aPKC phosphorylation site, a PSD95/Discs-large/ZO1 (PDZ)-binding motif and a 4.1/ezrin/radixin/moesin (FERM)-binding motif. Extensive studies in the past have demonstrated that the PDZ-binding motif interacts with PDZ domain in Pals1, a membrane-associated guanylate kinase (MAGUK) characterized by the presence of two
Lin-2/Lin-7 (L27) domains, a PDZ domain, a Src-homology 3 (SH3) domain and a guanylate kinase (GUK) domain, and functionlized in the maintenance of cell polarity[3, 14]. The FERM-binding motif is reported to bind to several FERM domain containing proteins like Expanded, an upstream regulators in Hippo pathway[15-17]; Yurt, a basolateral determinant negatively regulating the activity of Crumbs[18, 19]; Nevertheless, the detailed mechanism and connection of these interactions are largely unknown.

Ezrin/Radixin/Moesin (ERM) family is highly structural conserved characterized by the presence of an N-terminal FERM domain with ~300 amino acid, followed by a predicted coiled-coil region and C-terminal ERM-association domain (C-ERMAD), which is also the filamentous (F)-actin binding site. The FERM domain is consisted of three structural units (F1, F2 and F3) and together forms an organically clover-leafed supermodule. The C-terminal domain can fold back to strongly interact with FERM domain to form the so-called autoinhibition or inactive form. Phosphorylation at the conserved threonine residues in the actin-binding site (T567 in Ezrin, T564 in Radixin, T558 in Moesin) and/or regulation by phosphatidylinositol 4,5 biphosphates (PIP2) open the autoinhibition and turn the inactive ERM into active form[20-22]. Extensive work over the last two decades is mainly focused on the role of ERM proteins in organizing the cell cortex. Cell cortex is specialized membrane domains, such as the apical junctional domain, and is crucial in maintenance of cell shape and surface structures, cell adhesion, transportation of vesicles and cell polarity. ERM family is a key organizer of specialized domains in cell cortex by providing the linkage from F-actin filaments in the cytoskeleton to transmembrane proteins and receptors on the plasma membrane [23-25].

Moesin is one ERM gene in vertebrates and the single gene in drosophila (DMoesin). Except for its conventional role in stabilizing the cell cortex, more and more studies indicate its potential function in epithelial integrity and polarity. Moesin is localized to the apical surfaces of polarized cells, such as oocyte, imaginal discs and photoreceptors in drosophila. Among these polarized cells, absence of DMoesin leads to lethal phenotype and inactivation gives rise to significant alterations in morphogenesis. For example, during oogenesis, loss of Moesin function disrupts the actin filament attachment to the oocytes cortex and localization
of Oskar, the posterior determinants in oocyte [26, 27]. Also, the imaginal wing epithelia cells from Dmoesin mutant larvae migrate away from cell layers and no longer express epithelial marker E-cadherin, indicating a disruption of cell polarity[28]. Similar as in photoreceptors, inactivation of dMoesin impairs rhabdomere morphogenesis and disorganizes the actin filament of the apical region[29]. These mutant phenotypes are correlated to the abnormality in cell polarity under the downregulation of Crumbs, suggesting a possible linkage above transmembrane protein Crumbs and FERM-domain containing protein Moesin.

Here we identified the direct interaction of the cytoplasmic tail in Crumbs with the FERM domain in Moesin but not with the autoinhibited form, consistent with the hypothesis that activation of Moesin gives it access to sense the signal from transmembrane proteins and transduce signals to the underlying cytoskeleton and cytoplasm[22]. The crystal structure of the complex with FERM domain in Moesin and cytoplasmic tail in Crumbs is diffracted to 1.5 Å and shows a high resolution of the detailed binding region. Strikingly, other than binding to the conventional FERM-binging motif (GTY) in Crumbs, the Moesin FERM domain is also binding to the PDZ-binding motif (ERLI), implying a communication among different binging motifs underlying the Crumbs short cytoplasmic tail. More interestingly, the binding site that PDZ-binding motif occupies in Moesin is located at the F1/F3 cleft, which is the same as the site of inositol-1,4,5-trisphosphate (InsP3), the soluble head group of PIP2, suggesting that certain cues may prompt Crumbs to compete with PIP2 for the activation of Moesin or the specific binding of Crumbs and Moesin can exert special functions that PIP2 can not provide. The discovery of this novel binding mode strongly indicates that different binding motifs in Crumbs cytoplasmic tail are not separate but integrated as a whole. Moreover, this binding mode may also apply to other transmembrane proteins like Neurexin with their FERM domain containing partners Band 4.1 and assist to clarify the functional crosstalk between FERM domain containing proteins and PDZ domain containing proteins.
Chapter 2

Materials and Methods

Protein expression and purification.

The coding sequence of Crb-cyto (aa 2110-2146) was amplified from Drosophila Crumbs isoform A and cloned into modified pET32a vector and PGEX-4T-1 vector. Moesin FERM domain (aa 1-297) and C-ERMAD (aa 467-577) was amplified from cDNA library of muscle in mouse and cloned into modified pET32a vector. Proteins were expressed in BL21 (DE3) Escherichia coli cells at 16 °C overnight. The recombinant proteins were purified using Ni$^{2+}$-nitrilotriacetic acid agarose column or GSH-Sepharose column, followed by further step of size-exclusion chromatography.

GST Pull-Down Assay.

GST-tagged Crb-cyto (~ 1 nmol) in assay buffer (phosphate-buffered saline, pH 7.4) was incubated with GFP-tagged Moesin FERM domain and Merlin FERM domain for 2h at 4°C. After centrifuge, supernatant was loaded to 30 µl fresh GSH-Sepharose 4B beads for 0.5h. The pellets were washed three times with 1 ml assay buffer, followed by resolving of 15% SDS-PAGE and detected by western blot.

Analytical Gel Filtration Chromatography.

Proteins (50 µM, 200 µl) in assay buffer (50 mM Tris, 100 mM NaCl, 1 mM EDTA and DTT, pH 7.5) were loaded onto a Superose 12 10/300 GL column (GE Healthcare) equilibrated with same buffer.
Isothermal Titration Calorimetry (ITC) Assay.

ITC measurements were carried out on a VP-ITC calorimeter (MicroCal) at 25°C. Protein samples were in assay buffer with 50 mM Tris, 100 mM NaCl, 1 mM EDTA and DDT. The titration processes were performed by injecting 10 μl aliquots of Crb-CT into Moesin FERM domain with time intervals of 2 min to ensure titration peak returned to baseline.

Crystallization

Crystals of the mMoe-FERM/dCrbs-cyto complex were obtained by hanging drop vapor diffusion method at 16°C. To set up a hanging drop, 1 μl of concentrated protein mixture at 1:1 stoichiometric ratio was mixed with 1 μl of crystallization solution with 20% PEG3350 and 0.2 M ammonium iodine. Before diffraction experiments, crystals were soaked in crystallization solution containing 30% glycerol for cryoprotection. The diffraction data were collected at Shanghai Synchrotron Radiation Facility and were processed and scaled using HKL2000 [30].

Structure determination

The initial phase was determined by molecular replacement using the moesin FERM domain (PDB code: 1EF1) as the searching model. The model was refined in Phenix [31] against the 1.5 Å dataset. The dCrbs-cyto peptide was built subsequently in COOT [32]. In the final stage, an additional TLS refinement was performed in Phenix. The refinement statistics are listed in Table 1. All structure figures were prepared using PyMOL (http://pymol.sourceforge.net/).
Chapter 3

Results

3.1 The cytoplasmic tail of Crumbs specifically binds to the FERM domain in Moesin, but not the inactive form.

The FBM and PBM are highly conserved across species, including human and flies (Fig. 1). To investigate the Crumbs/Moesin interaction, we purified the cytoplasmic domain of Crumbs (Crbs-cyto) and the FERM domain of Moesin (Moesin-FERM) individually. As the quality of Moesin-FERM is not good enough in low concentration of salt (100 mM NaCl), we chose high concentration of salt (400 mM NaCl) to stabilize it. Meanwhile, as the Crbs-cyto is extremely conserved and binds to Moesin-FERM with a similar affinity across species (data not shown), we finally chose fly Crbs-cyto and mouse Moesin-FERM to do biochemical assay. Our GST pull-down assay showed that Crbs-cyto specifically binds to Moesin-FERM (Fig. 1B). The analytical gel-filtration results further prove that Crbs-cyto forms a stable complex with Moesin-FERM in solution (Fig.1C). The dissociation constant $K_d$ of the complex was measured to be $\sim 5$ μM by isothermal titration calorimetry (ITC)(Fig.1D). Adding Moesin C-terminal region (C-ERMAD) into the above complex affectively block the binding of Crbs-cyto to Moesin-FERM (Fig.1C), suggesting that activation of Moesin is required for the binding of its FERM domain to Crumbs. Consistent with the previous study, mutation of the strictly conserved Tyr2119 in the FBM of Crumbs totally disrupt the Crumbs/Moesin interaction (Fig. 1F), confirming that the FBM is a critical binding site for Moesin-FERM. Unexpectively, deletion of the PBM of Crumbs decreased the binding affinity by 3 folds (Fig.1E).
Fig. 1. Characterization of the Crbs-cyto/Moesin-FERM interaction. (A) The domain organization of Crumbs and Moesin. The sequence of Crumbs tail was aligned among species. The Crbs-cyto/Moesin-FERM interaction is indicated by an arrow. (B) GST pull-down assay showing that Crbs-cyto just binds to Moesin-FERM, not to Merlin-FERM. (C) Analytical gel-filtration analysis showing that Crbs-cyto and Moesin-FERM forms a 1:1 stoichiometric complex. No interaction between the Crbs-cyto and Moesin-FERM was detected after adding the Moesin C-ERMAD. (D-F) ITC based measurements of the binding affinities of Moesin-FERM with Crbs-cyto-WT (aa 2110-2146), Crbs-cyto-ΔPBM (aa 2110-2128), Crbs-cyto-Y2119A (GTY mutated to GTA).
3.2 Structure Determination of the Moesin/Crbs Complex

To understand the molecular basis governing the Moesin/Crbs interaction, we tried to determine the structure of the FERM domain of Moesin (Moe-FERM) in complex with the full cytoplasmic region of Crbs (Crbs-cyto) by using crystallography. By using the highly concentrated sample (30 mg/ml) of the Moe-FERM/Crbs-cyto complex, we obtained the high quality crystals diffracted to 1.5 Å resolution. The crystal structure was solved using molecular replacement method and subsequently refined with an $R$ and $R_{\text{free}}$ of 15.9 and 19.1, respectively (Table 1).
### Table 1. Statistics of data collection and model refinement

<table>
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<tr>
<td>Space group</td>
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<tr>
<td>Unit cell parameters (Å)</td>
<td>$a = 63.2$, $b = 65.2$, $c = 83.4$</td>
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<tr>
<td>Resolution range (Å)</td>
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<tr>
<td>No. of unique reflections</td>
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<td>Redundancy</td>
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<tr>
<td>$I/\sigma$</td>
<td>33.5 (3.5)</td>
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<tr>
<td>Completeness (%)</td>
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<td>$R_{merge}$ (%)$^a$</td>
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<tr>
<td>Resolution (Å)</td>
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$^a R_{merge} = \sum |I_i - I_m| / \sum I_i$, where $I_i$ is the intensity of the measured reflection and $I_m$ is the mean intensity of all symmetry related reflections.

$^b R_{cryst} = \sum ||F_{obs}|| / ||F_{calc}||$, where $F_{obs}$ and $F_{calc}$ are observed and calculated structure factors.

$^c R_{free} = \sum_T ||F_{obs}|| - ||F_{calc}|| / \sum_T ||F_{obs}||$, where $T$ is a test data set of about 3.6% of the total.
reflections randomly chosen and set aside prior to refinement.

c Defined by MolProbity.

Numbers in parentheses represent the value for the highest resolution shell.
3.3 Overall Structure of the Moe-FERM/Crbs-cyto complex

Consistent with our biochemical data, Moe-FERM and Crbs-cyto forms a heterodimer in the crystal structure with one complex per asymmetric unit. The final structural model contains most of residues from the complex, except for a flexible loop (residues 2127-2135) of Crbs-cyto, which connects the FBM and PBM (Fig. 2). Like other FERM domains in ERM proteins, Moe-FERM adopt a typical architecture, comprised of three lobes, F1, F2, and F3 (Fig. 2&3). F1, F2, F3 are structurally similar to ubiquitin, acyl-CoA-binding protein and plekstrin-homology (PH) domain, respectively, and arranged as a cloverleaf[20]. The overall fold of Moe-FERM in the complex structure is similar with the free Moesin FERM structure (the overall RMSD of 1.6 Å with 285 aligned residues). The two highly conserved motifs in Crbs-cyto, the FBM and PBM bind with Moe-FERM at the F3 lobe and a cleft between the F1 and F3 lobes, respectively (Fig. 2).
Fig. 2. Overall structure of the Moe-FERM/Crbs-cyto complex. The crystal structure is present as ribbons diagram with the three lobes of Moe-FERM, F1 (green), F2 (cyan), and F3 (blue), and the two motifs of Crbs-cyto, FBM (orange) and PBM (pink) drawn in their specific colors. The same color code is used throughout the rest of the figures. The disordered loop connecting FBM and PBM is indicated by a dotted line.
Fig. 3. Sequence alignment of the FERM domains from ERM family members. In this alignment, residues that are absolutely conserved and highly conserved are highlighted in red and yellow, respectively. The secondary structural elements are indicated above the alignment and the coloring scheme matches with the structure of the protein shown in Figure 2. The residues involved in the binding of Moe-ERM to the FBM and to the PBM are indicated by solid circles and triangles, respectively.
3.4 The FBM of Crumbs binds to the typical F3 binding site

In FERM-containing proteins, F3 lobes act as the major target binding sites. A groove (termed αβ-groove) mainly formed by β5F3 and α1F3 is a well-characterized binding region in FERM domains including those of moesin [20], radixin [33-37], talin [38-40], and myosin-X [41, 42]. The most targets, especially those of ERM proteins, bind to the αβ-groove in a β-strand or β-strand like extended structures (Fig. 4B). In the Moe-FERM/Crbs-cyto complex, the FBM of Crumbs also adopts a β-strand structure and binds to the αβ-groove in the F3 lobe of Moe-FERM (Fig. 4A), extending the anti-parallel β-sheet formed by β5F3, β6F3, and β7F3. The FBM/F3 interaction is mainly mediated by H-bonds, including a dozen of mainchain-mainchain, sidechain-mainchain, and sidechain-sidechain H-bonds, which forms an extensive H-bond network to stick the FBM to the αβ-groove (Fig. 4A). Some hydrophobic interactions (e.g. Pro2121Crbs inserts its aliphatic sidechain into a hydrophobic cave formed by Ile245F3 and Ile248F3) also contribute to the FBM/F3 interaction (Fig. 4A). The three continuing and strictly conserved residues in the FBM, Gly2117, Thr2118, and Tyr2119 (termed as ‘GTY’ motif, which is usually regarded as a sequence icon for FBM) are invariably involved in the FBM/F3 interaction using their unique amino acid properties (Fig. 4A). As lacking of sidechain, Gly2117 tightly packs with the ring of Phe250F3 without introducing steric hindrances. Thr2118 is H-bonded to Asp247F3 via its sidechain. Tyr2119 not only forms a strong H-bond (bond length of 2.7 Å) with His288F3, but also has a hydrophobic interaction with Met285F3. Consistent with our structural findings, the Tyr2119Ala mutation, which disrupt both the H-bonds and hydrophobic interaction, abolished the Moesin/Crumbs interaction (Fig. 1E).
Fig. 4. The FBM/F3 interaction. (A) The molecular details are shown for the FBM/F3 interface. The salt bridges and hydrogen bonds are indicated as dashed lines. The sidechain of Ser2120Cbs adopts two alternative conformations, either of which forms a H-bonds with residues in the F3 lobe. (B) Two examples showing that targets (CD44 and ICAM-2) bind to the αβ-groove in the F3 lobe of Radixin (an ERM protein) in a β-strand structures.
3.5 The PBM of Crumbs binds to the F1/F3 cleft

Although the FBM is required for the Moesin/Crumbs interaction, our ITC data indicates that the PBM is also involved in this interaction (Fig. 1F). Consistently, the Moe-FERM/Crbs-cyto structure shows that the PBM of Crumbs directly interact with Moe-FERM (Fig. 1&5). Strikingly, unlike any other structurally characterized protein target binding modes of FERM-containing proteins, the PBM resides deeply on the cleft between the F1 and F3 lobes (Fig. 6). This novel binding site consists of highly conserved residues from both F1 and F3 lobes. The PBM interacts with these residues through charge-charge interactions, H-bonds, as well as hydrophobic interactions. Interestingly, the C-terminal last four residues, which is a typical PDZ binding sequence and is critical for the binding of Crumbs to Pals1, are involved in the Moe-FERM/Crbs-cyto interaction (Fig. 5). The C-terminal carboxyl group of Crumbs forms a salt bridge with Lys278 of the F3 lobe and Glu2143Crbs forms two salt bridges with Lys60 and Lys83 of the F1 lobe, respectively. Leu2145Crbs packs with a hydrophobic pocket formed by Leu281, Phe250, and the aliphatic part of Lys278 in the F3 lobe. The residues immediately N-terminal to the last four residues of Crbs-cyto also contributes to the binding of the PBM to the F1/F3 cleft (Fig. 5). Glu2142Crbs forms two H-bonds with the mainchain atoms of Leu61 and Asn62 in the β4/β5 loop of the F1 lobe. The two tandem proline residues, Pro2140Crbs and Pro2141Crbs are involved in hydrophobic interactions with the residues from the F1 and F3 lobes.

We note with interest that the residues involving the Moe-FERM/Crbs-cyto interaction are all highly conserved in ERM proteins (Fig. 3&6) and in Crumbs homologs (Fig. 1) across species, suggesting that this interaction appears at low eukaryotes (e.g. flies and worms), which only contain one ERM protein, and is retained and expand to three ERMs and three Crumbs in mammals during evolution.

As a well-known membrane binding protein, ERM proteins were found to bind with not only cytoplasmic domains of numerous membrane proteins, but also plasma membrane by interacting with the headgroup (InsP₃) of phosphatidylinositol 4,5-bisphosphate (PI₃P). The upper part of the F1/F3 cleft is the InsP₃ binding site (Fig. 7B). Interestingly, the site is
occupied upon Crbs-cyto binding (Fig 7A), indicating that the bindings of Moesin to Crumbs and PIP$_2$ are mutually exclusive.
Fig. 5. The molecular details are shown for the interaction between the PBM of Crbs-cyto and the F1/F3 cleft of Moe-FERM. The salt bridges and hydrogen bonds are indicated as dashed lines.
Fig. 6. Surface distribution of conserved residues in Moe-FERM. Surface residues that are absolutely conserved, highly conserved, and not conserved according to the sequence alignment in Figure 3, are colored in green, light green, and white, respectively. Both the FBM and PBM binding sites in Moe-FERM are highly conserved.
Fig. 7. The binding of Crumbs (A) and InsP₃ (B) to Moe-FERM are interfered with each other. These two bindings share two interacting lysine residues, K63 and K278.
Chapter 4

Conclusion and Discussion

We discover in this study that PDZ-binding motif (PBM) is involved in the interaction with FERM domain. Analysis of the structure shows that F1/F3 cleft in FERM domain is suitable for occupying the PDZ-binding motif, which is the same site that PIP2 occupies. To confirm the novel two binding sites, we mutate the FERM-binding motif (GTY to GTA) or delete the PDZ-binding motif (ΔERLI), the binding affinity is obviously decreased, indicating the two sites are both contributed to the binding affinity. Analysis from the crystal structure, we find that charge-charge interactions contribute some affinity to the FBM site, while no charge-charge interactions are found in PBM site. This explains the affinity constant Kd of Crumbs-cyto-WT with Moesin-FERM is just 5 μM as we measure the affinity in buffer with 400 mM NaCl, which may disrupt some charge-charge interactions. Based on the above information, it is clear that FBM is the major site which determines the binding affinity, yet the PBM is the other site determining the binding specificity.

Despite the high sequence similarity between FERM domain in Moesin and Merlin, the Merlin FERM domain is unable to bind to Crumbs cytoplasmic tail, indicating certain binding specificity among FERM domains with transmembrane proteins. Except for this, the Merlin C-terminus lacks the canonical actin-binding region existed in all ERM proteins. Considering these differences, it is not surprising that the localization and function of Moesin and Merlin is distinct from each other. However, the key question that how Merlin provides linkage between plasma membrane and cytoskeleton is still unsolved. Our structure may probably help explain these differences, although further experiments need to be designed.

Similar as Crumbs, a series of transmembrane proteins and cell adhesion molecules such as Neurexin, SynCAM and Syndecan all integrate FERM binding motif and PDZ binding motif together. From evolution angle, this integration must have certain meanings, probably
building a bridge between cell cortex and cell polarity. Referring to our complex structure, it is reasonable to believe that Neurexin, SynCAM, Syndecan and other similar molecules bind to their respective FERM domain proteins with the same mode. If this is a general mechanism, it will be helpful to explain the functional linkage of FERM domain containing proteins with transmembrane proteins, thereby providing a signal platform for plasma membrane and cell cortex. Meanwhile, as the PDZ-binding motif also binds to a cascade of PDZ domain containing proteins with different affinities, how PDZ-binding motifs regulating these various PDZ domain containing proteins will also be an exciting area. Moreover, understanding how PDZ domain containing proteins crosstalk with FERM domain containing proteins when binding to the transmembrane or cell adhesion molecules will make a significantly contribution to this field, as the connection of plasma membrane, cell cortex and cell polarity will be functionally established
Reference


33. Hamada, K., et al., Structural basis of adhesion-molecule recognition by ERM proteins


