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Self-masking in an Intact ERM-merlin Protein: An Active Role for the Central α-Helical Domain

Qianzhi Li[1], Mark R. Nance[2], Rima Kulikauskas[3], Kevin Nyberg[3] Richard Fehon[3], P. Andrew Karplus[4], Anthony Bretscher[5] and John J. G. Tesmer[2]*

[1]*Department of Chemistry and Biochemistry, Institute for Cellular and Molecular Biology The University of Texas at Austin 1 University Station #A5300, Austin TX 78712-0165, USA*

[2]*Life Sciences Institute Department of Pharmacology 210 Washtenaw Ave. University of Michigan Ann Arbor, MI 48109, USA*

[3]*Department of Molecular Genetics and Cell Biology University of Chicago, Chicago IL 60637, USA*

[4]*Department of Biochemistry and Biophysics, Oregon State University, Corvallis OR 97331, USA*

[5]*Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853 USA*

**Corresponding author*

Ezrin/radixin/moesin (ERM) family members provide a regulated link between the cortical actin cytoskeleton and the plasma membrane to govern membrane structure and organization. Here, we report the crystal structure of intact insect moesin, revealing that its essential yet previously uncharacterized α-helical domain forms extensive interactions with conserved surfaces of the band four-point-one/ezrin/radixin/moesin (FERM) domain. These interdomain contacts provide a functional explanation for how PIP_2 binding and tyrosine phosphorylation of ezrin lead to activation, and provide an understanding of previously enigmatic loss-of-function missense mutations in the tumor suppressor merlin. Sequence conservation and biochemical results indicate that this structure represents a complete model for the closed state of all ERM-merlin proteins, wherein the central α-helical domain is an active participant in an extensive set of inhibitory interactions that can be unmasked, in a rheostat-like manner, by coincident regulatory factors that help determine cell polarity and membrane structure.

Keywords: structure; actin; ERM; merlin; coiled-coil

Introduction

The plasma membrane is organized into functional regions, with the distinct apical and basolateral domains of polarized epithelial cells providing a well-studied example. To assemble, maintain and regulate the composition and structure of these domains, eukaryotes have evolved proteins that link the underlying cytoskeleton to specific membrane proteins. Among the best understood class are the ezrin/radixin/moesin (ERM) family, which provide a conformationally regulated linkage from the cortical actin cytoskeleton to the plasma membrane, especially in structures like microvilli.[1,2]

ERM proteins consist of three principal domains (Figure 1(a)). The best characterized of these are the band four-point-one/ezrin/radixin/moesin (FERM) domain[3] and the C-terminal tail domain. The N-terminal ~300 residue FERM domain consists of three lobes, designated F1, F2 and F3, that are tightly associated in a cloverleaf-like structure.[4] The FERM domain of ERM proteins binds directly to integral membrane proteins, such as CD43, CD44 and ICAM1-3, through their positively charged juxtamembrane regions,[5–9] or indirectly through

Abbreviations used: ERM, ezrin/radixin/moesin; FERM, band four-point-oneERM; *Sf*moesin, moesin from *Spodoptera frugiperda*.

E-mail address of the corresponding author: johntesmer@umich.edu

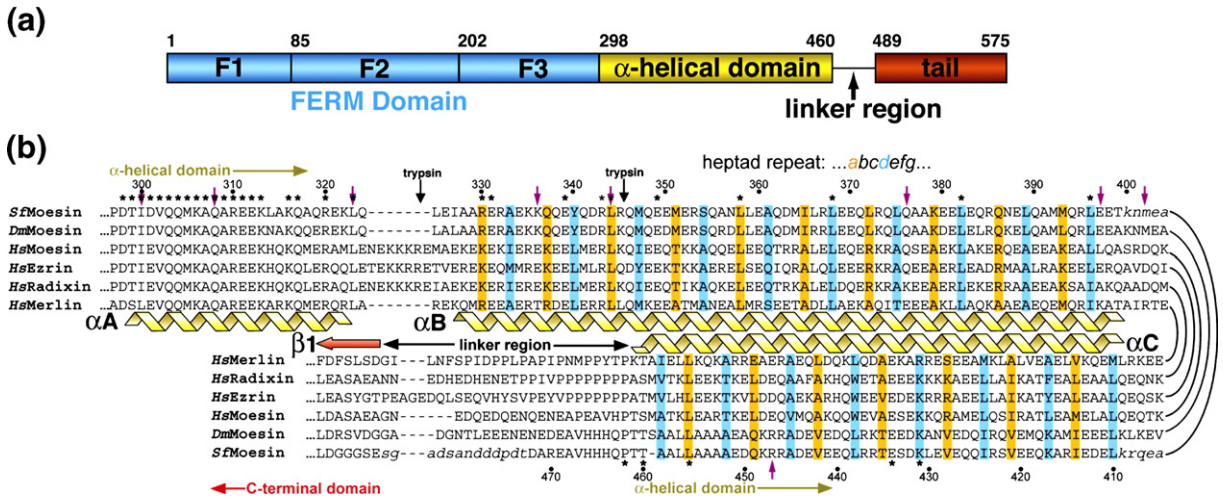

Figure 1. (a) Domain structure of *Sf*moesin. Residue numbers at the domain boundaries are indicated. (b) Alignment of ERM-merlin α-helical domains. The sequence for αC is folded back (runs right to left) to indicate its register with the αB helix. Helical regions are indicated by a yellow coil, and the β1 strand of the C-terminal tail by a red arrow. The *a* and *d* positions of the coiled-coil heptad repeat are shown with orange and cyan backgrounds, respectively.[16] These positions interact with the other helix of the coil, as shown. Residues that are disordered in both *Sf*moesin structures are shown with lower case italics. Sequence numbering corresponds to that of *Sf*moesin, and the asterisks indicate invariant or highly conserved residues. Sites sensitive to trypsin digestion in the radixin α-helical domain (black arrows), and positions in human merlin associated with cancer (purple arrows) are indicated. Sequences used are as follows: human merlin (*Hs*Merlin), SwissProt accession no. P35240; human radixin (*Hs*Radixin), P35241; human ezrin (*Hs*Ezrin), P15311; human moesin (*Hs*Moesin), P26038; and *D. melanogaster* moesin (*Dm*Moesin), GenBank accession no. NP_996392.

the PDZ-containing scaffolding proteins EBP50/NHERF and E3KARP.[10–12] The C-terminal tail domain (often referred to as the C-terminal tail or C-terminal ERM association domain or C-ERMAD) spans the last ~100 residues and contains an F-actin binding site in the last 30 residues.[13–15] This domain interacts with the FERM domain as an extended, meandering polypeptide beginning with a β-strand associated with β5 in F3 followed by four helices, the first two of which bind lobe F2 and second two of which bind lobe F3 (Figure 2(a)).[4] The FERM-tail complex represents a dormant form of the protein in which membrane protein and active binding sites are masked.

Linking the FERM and C-terminal domains is an essential but structurally uncharacterized domain of ~190 residues, referred to as the α-helical domain, the most conserved feature of which being a heptad repeat characteristic of α-helical coiled-coils (Figure 1(b)).[16] This region has been proposed to form an extended helical tether in activated ERM proteins linking the membrane-binding determinants of the FERM domain to the actin-binding determinant at the C-terminal tail.[17] Although crystal structures have been reported for activated FERM domains as well as for the inactive FERM domain complexed with the C-terminal tail domain of human moesin, they have revealed at most only a small portion of the important and enigmatic α-helical domain.

Equally enigmatic is the neurofibromatosis 2 (NF2) tumor suppressor protein merlin, which is closely related to ERM proteins and shares all of the above features except for actin binding.[18,19] Mutations in merlin that lead to loss of tumor suppression are often disruptive, either truncating the protein or interfering with the proper fold of the protein. Some missense mutations of merlin associated with NF2 have been mapped to the interface of the FERM and C-terminal tail domain,[4] suggesting that their association is critical for tumor suppressor activity. However, many more mutations remain unexplained, given the currently available models.

A particularly interesting aspect of ERM proteins is that they can exist in at least two conformational states,[1] an active open form with the FERM and C-terminal tail domain dissociated, and a dormant closed form similar to that described for the human moesin FERM-C-terminal tail domain complex.[4] Dissociation of the FERM and C-terminal tail domains unmasks binding sites for other proteins. EBP50 binds to a region of the FERM domain that overlaps with the C-terminal tail,[20,21] and the ICAM-2 receptor binds to the radixin FERM domain at a site analogous to the first β-strand in the C-terminal tail.[22] This site is analogous to where integrin tails anchor to the talin FERM domain,[23] and to where a regulatory intramolecular linker region interacts with the FERM domain of focal adhesion kinase.[24] Evidence suggests these states are regulated primarily by the phosphorylation of a threonine residue in the C-terminal domain (equivalent to Thr558 in moesin) and/or the binding of PIP_2.[1] Thr558 lies buried in the FERM–C-terminal tail interface, and its phosphorylation is expected to favor domain dissociation. PIP_2 binds to a site between lobes F1 and F3, and has been proposed to confer subtle conformational changes that favor