Molecules in focus

**Ezrin/radixin/moesin: Versatile controllers of signaling molecules and of the cortical cytoskeleton**

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**Abstract**

Ezrin, radixin and moesin (ERM) proteins are widely distributed proteins located in the cellular cortex, in microvilli and adherens junctions. They feature an N-terminal membrane binding domain linked by an α-helical domain to the C-terminal actin-binding domain. In the dormant state, binding sites in the N-terminal domain are masked by interactions with the C-terminal region. The α-helical domain also contributes to masking of binding sites. A specific sequence of signaling events results in dissociation of these intramolecular interactions resulting in ERM activation. ERM molecules have been implicated in mediating actin–membrane linkage and in regulating signaling molecules. They are involved in cell membrane organization, cell migration, phagocytosis and apoptosis, and may also play cell-specific roles in tumor progression. Their precise involvement in these processes has yet to be elucidated.

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**Keywords:** ERM; Actin–membrane linkage; Membrane organization; Cell migration

1. **Introduction**

Reversible actin–membrane linkage is essential for maintenance of cell shape, for cell adhesion, migration and division. Ezrin (cytovillin), radixin and moesin (ERM) proteins are closely related proteins linking actin filaments to the membrane either (i) directly via binding to cytoplasmic tails of transmembrane proteins or (ii) indirectly via scaffolding proteins attached to transmembrane proteins. ERM proteins were originally characterized 20 years ago as structural components of the cell cortex, localized in microvilli and adherens junctions. Recent studies in mice suggest redundant functions of the three proteins (Bretscher, Edwards, & Fehon, 2002; Fiévet, Louvard, & Arpin, 2007). For lack of space this review will not discuss the putative tumor suppressor merlin, an ERM-related protein.

2. **Structure**

The gene for ezrin (13 exons) has been mapped to chromosome 6, that of moesin to chromosome X and that of radixin to chromosome 11. The closely related ERM proteins were originally characterized 20 years ago as structural components of the cell cortex, localized in microvilli and adherens junctions. Recent studies in mice suggest redundant functions of the three proteins (Bretscher, Edwards, & Fehon, 2002; Fiévet, Louvard, & Arpin, 2007). For lack of space this review will not discuss the putative tumor suppressor merlin, an ERM-related protein.

**Abbreviations:** C-ERMAD, C-terminal ERM association domain; ERM, ezrin, radixin, moesin; FERM, four-point one ERM; GDI, guanine nucleotide dissociation inhibitor; MRP2, multidrug resistance protein 2; N-ERMAD, N-terminal ERM association domain; NIK, Nck-interacting kinase; P(4,5)P2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C

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Fig. 1. Domain structure of ERM proteins. (A) Comparison of the domain organization and sequence identity between human ERM proteins (NCBI accession numbers: ezrin, NP 003370; radixin, NP 002897; moesin, NP 002435). CTD, C-terminal domain; P, polyproline domain. The F-actin-binding site is located within the last 30 residues at the C-terminus of the ERM proteins (Bretscher et al., 2002). (B) Ribbon structure of dormant moesin from Spodoptera frugiperda (3.0 Å structure; Protein Data Bank accession number 2I1K; Li et al., 2007). Lobes F1, F2 and F3 of the FERM domain are colored yellow and the C-terminal domain in red. The α-helical domain (blue) folds into three extended helices (αA, αB and αC), containing elements that contact the FERM domain. The linker between the α-helical domain and the C-terminal domain (amino acids 461–472) colored in violet interacts mainly with lobe F1 of the FERM domain. The C-terminal domain associates with lobes F2 and F3 of the FERM domain. Backbones of conserved amino acids implicated in interaction with PI(4,5)P2 are colored black (from top to bottom: lysines 64, 63, 60, 278, arginine 253, lysines 254, 262, 263). The following parts of the molecule are not observed in the crystal (dashed black lines): the αB–αC loop of the α-helical domain and the connection between the linker (violet), and the beginning of the C-terminal domain.

a tri-lobed structure. This domain is connected via a central α-helix-rich domain to the C-terminal domain which contains a major F-actin-binding site, enabling these proteins to link actin filaments to the plasma membrane. In the absence of activating signals, ERM proteins are maintained in an inactive conformation through intramolecular interactions between the N-terminal ERM association domain (N-ERMAD) and the C-terminal ERM association domain (C-ERMAD), masking membrane and F-actin-binding sites (Fig. 1A and B). Recent data by Li et al. (2007), based on the first crystal structure of intact insect moesin in the closed state, show that interactions of the central α-helix-rich domain and linker regions with lobes F1 and F2 of the FERM domain also importantly contribute to masking of binding sites, especially those for phosphoinositides (Fig. 1B) (Bretscher et al., 2002; Fièvet et al., 2007; Li et al., 2007).

3. Expression and activation

Genes encoding for ERM proteins have been identified in all multicellular metazoan organisms analyzed so far. The three isoforms are widely distributed in vertebrates, whereas other species only express one isoform. In mice, most tissues co-express all three isoforms in variable expression ratios, but the liver and intestine lack ezrin and radixin expression, respectively. Ezrin is enriched at the apical side of epithelial cells, moesin is a major isoform in endothelial cells and leukocytes, and radixin is especially abundant in liver (Doi et al., 1999).

The activation state of ERM proteins is tightly regulated by signaling. Binding of the protein to the membrane lipid phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and subsequent phosphorylation of a conserved C-terminal threonine are thought to disrupt the intramolecular associations, thus unmasking sites involved in interaction with other proteins (Fig. 2A). Based on crystallization studies and site-directed mutagenesis, the binding site for PI(4,5)P2 has been located in a basic groove between subdomains F1 and F3 involving conserved, positively charged amino acids contributed from both lobes (Barret, Roy, Montcourrier, Mangeat, & Niggli, 2000; Hamada, Shimizu, Matsui, Tsukita, & Hakoshima, 2000) (Fig. 1B). According to the crystal
Fig. 2. Activation and cell-type dependent functions of ERM proteins. (A) For stable activation, ERM proteins in a first step interact via their N-terminal FERM domain with the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2), followed by C-terminal phosphorylation on a conserved threonine residue. The stably activated protein can then link actin filaments (F-actin) to single pass or multiple pass membrane proteins, or interact for example with GDI proteins that regulate functions of small GTP-binding proteins. F1, F2 and F3 correspond to the three lobes of the FERM domain. ROCK, Rho-kinase; β2AR, β2-adrenergic receptor; CFTR, cystic fibrosis transmembrane conductance regulator; ICAM-1, intercellular adhesion molecule 1. For the other abbreviations see text. (B) Localization and putative functional roles of ERM proteins in different cell types. IS, immunological synapse; APC, antigen-presenting cell. For details see text.
Based on the findings of Li et al. (2007), phosphorylation of Tyr353 in ezrin would be expected to induce release of the α-helical domain from the FERM domain.

4. Biological functions

ERM proteins interact via their FERM domains with three types of proteins: transmembrane proteins, scaffolding proteins that bind to transmembrane receptors and signaling molecules such as phosphatidylinositol 3-kinase or a Rho-specific guanine nucleotide dissociation inhibitor (GDI) (Fig. 2A; Bretscher et al., 2002; Ivetic & Ridley, 2004; Loebrich, Bähring, Katsuno, Tsukita, & Kneussel, 2006). These findings predict a role for ERMs in organizing transmembrane receptors and in signal transduction. Data obtained using knockout mice lacking individual ERM isoforms especially support a membrane-organizing role of ERM proteins. For each of the ERM proteins, knockout mice have been generated. The data obtained with these mice mostly support the functional redundancy of the three proteins. Defects were only observed for those organs where only one of the ERM proteins was expressed.

A lack of ezrin, the only isoform expressed in part of the polarized epithelia of the intestine, results in abnormal villus morphogenesis and a disrupted terminal web in these cells. As a consequence, neonate mice lacking ezrin fail to thrive and do not survive past weaning (Saotome, Curto, & McClatchey, 2004). Using a knockdown approach in mice, a role of ezrin has been demonstrated in formation/expansion of canicular api- cal membranes in gastric parietal cells, which express only ezrin, resulting in achlorhydria. This work suggests ERM proteins play a role in vesicle trafficking (Tamura et al., 2005).

Radixin is the dominant ERM protein in hepatocytes and the only detectable isoform present in stereocilia of the cochlea of adult mice. Mice lacking radixin develop normally but show mild liver injury after 8 weeks past birth. Radixin appears to mediate localization of the multidrug resistance protein 2 (MRP2) in bile canicular membranes via a direct interaction. MRP2 is involved in secretion of conjugated bilirubin into bile (Kikuchi et al., 2002). The lack of radixin also results in deafness in mice. This is due to defective stereocilia in the inner and outer hair cells, which normally exclusively express radixin. No defects are observed in vestibular stereocilia, where the small amounts of ezrin expressed appear to compensate for the lack of radixin (Kitajiri et al., 2004). This is a clear-cut example for the functional redundancy of radixin and ezrin in vivo. Mutations in human radixin also correlate with neurosensory hearing loss (Khan et al., 2007). Interestingly, radixin appears to specifically mediate anchoring of the GABA<sub>A</sub> receptor α5 subunit to the actin cytoskeleton, dependent on radixin-phosphoinositide binding and activation. GABA<sub>A</sub> receptor clustering is not observed in hippocampal slices of mice lacking radixin. Ezrin and moesin in contrast do not appear to interact with this receptor (Loebrich et al., 2006). This is one of the first studies suggesting ERM proteins have an isoform-specific function.

Moesin knockout mice show no apparent defects. This is surprising as moesin shows some structural differences from ezrin and radixin, lacking a proline stretch present in the two other proteins, and as moesin is not phosphorylated on tyrosine (Doi et al., 1999).

Functions of ERM proteins appear to differ depending on the cell type (Fig. 2B). In leukocytes, ERMs may control signaling molecules such as the small GTP-binding protein Rho and the enzyme phosphatidylinositol 3-kinase. ERMs are required for formation and maintenance of microvilli and modulation of important functions such as clustering of cell adhesion molecules in lymphocyte uropods, tail retraction of migrating cells, phagocytosis, T cell apoptosis and so forth (Fig. 2B) (Charrin & Alcover, 2006; Ivetic & Ridley, 2004). However, the molecular role of ERM proteins in these functions is unclear, particularly their involvement in cell migration, for which conflicting data exists. Observations in neutrophils and T lymphocytes suggest that ERM inactivation may be required for tail retraction and cell polarization. In contrast to these findings, expressing activated phosphomimetic ezrin in a spontaneously polarized T lymphoma cell line, which exhibits enrichment of P-ERM in the uropod, enhances uropod size and chemotaxis (Charrin & Alcover, 2006; Ivetic & Ridley, 2004; Lee et al., 2004). In a rat tumor cell line (most likely monocytoid in origin), attenuation of ERM expression does not affect cell polarization, but significantly reduces the fraction of spontaneously migrating cells. This finding cannot be explained by requirement of ERM proteins for Rho activation, as Rho activity was not impaired by ERM depletion (Rossy, Gutjahr, Blaser, Schlicht, & Niggli, 2007). ERM proteins may thus have cell-type specific functions in polarization and migration.

Concerning the role of ERM isoforms in signaling, they may act as scaffolding proteins, recruiting for example Ras into a complex with its activator at the membrane upon receptor occupancy (Orian-Rousseau et al., 2007). ERM proteins could also regulate signaling by modulating organization of lipid rafts which are thought to
be platforms for organization of signaling molecules. In resting B lymphocytes, ezrin appears to link lipid rafts to the cortical actin network. Transient activation-dependent dissociation of ezrin from lipid rafts appears to be a trigger for the release of lipid rafts from the cortical actin network, allowing stimulus-dependent lipid raft coalescence (Gupta et al., 2006).

5. Possible medical application

In two pediatric tumors, ezrin overexpression has been shown to correlate with clinical stage. Concomitantly, reduction of ezrin expression in rhabdomyosarcoma or osteosarcoma cell lines results in a decrease in pulmonary metastases in mice. In these cell lines, ezrin may contribute to metastasis by suppressing apoptosis, disturbing cell–cell adhesion and activating Rho. Depending on the type of adult human tumor, loss or upregulation of ezrin correlates with poor prognosis indicating cell-specific functions of ezrin in tumor progression (Hunter, 2004). As ERM proteins have been implicated in Ras signaling (Orian-Rousseau et al., 2007), uncontrolled Ras activation via ERM proteins could for example promote tumor progression.

6. Open questions

Concerning structure–function relationship, crystal structures of intact dormant and activated ERM molecules are required for the full understanding of the molecular events during activation. The binding site for phosphoinositides should be defined more precisely, as well as the conformational changes resulting from its occupancy and from phosphorylation of the C-terminal threonine. Information on specific protein binding sites (except for C-ERMAD-N-ERMAD interactions) is yet lacking. It is also not clear whether ERM proteins can interact simultaneously with several interaction partners.

Concerning biological functions, the precise functions of ERM proteins in cell polarization, tail retraction and migration will have to be elucidated using downregulation of specific isoforms. Targeted disruption of all three ERM proteins in tissues is required for elucidation of their in vivo functions.

More data on the role of ezrin and the other ERM proteins in human tumor development and metastasis are required to decide, whether the role of ERM proteins in these processes varies depending on the tissue, whether different isoforms play different roles and whether their role in tumor progression can for example be explained by their impact on Ras signaling.

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