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Structure and Functions: ERM Protein Family

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Abstract: Preservation of the structural integrity of the cell depends on the plasma membrane in eukaryotic cells. Interaction between plasma membrane, cytoskeleton and proper anchorage influence regular cellular processes. The needed regulated connection between the membrane and the underlying actin cytoskeleton is therefore made available by the ERM (Ezrin, Radixin, and Moesin) family of proteins. ERM proteins also afford the required environment for the diffusion of signals in reactions to extracellular signals. Other studies have confirmed the importance of ERM proteins in different mode organisms and in cultured cells to emphasize the generation and maintenance of specific domains of the plasma membrane. An essential attribute of almost all cells are the specialized membrane domains. They are specifically important to tissues like the intestinal brush border epithelium, with a highly organized cell cortex including a compound array of apical microvilli, an apical junctional complex, and a basolateral membrane domain. This paper critically looks at the structure and functions of the ERM proteins and briefly presents the activation and deactivation mechanism through careful analysis on works done on this protein and its prospects. It is obvious from the discussion presented in this paper that the ERM (Ezrin, Radixin, and Moesin) proteins play very vital roles in mediating signal transduction and maintaining cellular integrity from a variety of extracellular inputs through their interaction with different receptor tyrosine kinases (RTKs) such as EGFR and HGFR, adhesion and adaptor proteins such as E-cadherin, ICAM-1,2,3, NHERF and CD44, and other signaling pathways such as PI3K/Akt, cAMP/PKA and the Rho GTPases, all of which have been implicated in tumorigenesis; thus, making ERM proteins a crucial target in development of novel therapeutics in fighting cancer progression and other related disease conditions where the protein is implicated. Further analysis on the structure and reaction mechanism of this protein is needed to exploit its full potential for clinical and other uses.

Keywords: Cytoskeleton, Ezrin, Radixin, Moesin, Basolateral, Membrane, Domain

1. Introduction

Cellular structures such as filopodia, lamellipodia, apical microvilli, and ruffling membranes, cleavage furrow of mitotic cells, retraction fibers, and adhesion sites may contain ERM proteins where plasma membrane interacts with F-actin. The ERM protein family is made up of Ezrin, radixin and moesin (Figure 1), which are three closely related proteins [1, 2]. Vertebrates have three paralogs, ezrin, radixin and moesin present, while other species have only one ERM gene present. As such, it is likely that these paralogs in vertebrates arose by gene replication throughout evolution, ERM proteins are highly preserved [3]. The N-terminal and C-terminal of vertebrates (ezrin, radixin, and moesin), Drosophila (dmoesin) and C. elegans (ERM-1) homologs preserve more than 75% identity. For structural stability and for maintaining the integrity of the cell cortex by coupling transmembrane proteins to the actin cytoskeleton, ERMs are vital [4]. These proteins also play very critical intracellular scaffolding functions that help in signal transduction between

the intracellular and extracellular compartments of the cell as well as interacting with other membrane phospholipids. Regulating several cellular processes including reorganization of actin cytoskeleton, cell survival, membrane dynamics, cell migration, adhesion and regulation of membrane protrusion is what ERMs are involved in [5].

Potentially found in different chromosomes, ERM proteins are extremely similar. They have an N-terminal FERM/N-ERMAD domain, responsible for the interaction with numerous proteins, and a C-terminal domain (C-ERMAD) that binds the actin cytoskeleton. Flexible domain rich in α-helices that can form a coiled coil structure that allows inter- or intramolecular interactions between N- and C-ERMADs and links the N- and C-terminal domains. In their closed confirmation, ERM proteins are inactive [6]. Key for triggering the protein is a threonine residue (Thr-558 in moesin, Thr-576 in ezrin and Thr-564 in radixin) in the C-ERMAD domain. The interaction between N- and C-ERMAD domains is abrogated, and both domains are able to act together with cognate proteins and F-actin respectively if the ERM proteins is phosphorylated [7]. Although the crystallization of the *S. fugiperda*moesin cast some doubts about this mechanism, binding of phosphatidyl-inositol 4, 5-bisphosphate to the ERM protein activates it. Rho kinase, protein kinase Cα and θ, NF-κB-inducing kinase (NIK; also known as MAP3K14), MST4 and lymphocyte-oriented kinase (LOK; also known as STK10) are different types of kinases that have been shown to phosphorylate the regulatory threonine. Once activated, the ERM proteins interact with a variety of transmembrane proteins [8, 9].

The ERM binding motif of transmembrane proteins in CD44 and other cell surface receptors was first studied by Yonemura et al (1998). A cluster of basic amino acids located in the juxta-membrane CT domain was delineated by these authors [10]. Although these clusters are obviously involved in binding, more refined X-ray crystallographic studies have shown that the binding motif is a non-polar region flanked by N- or C-terminal basic regions. A detailed study by Hamada et al., 2003 indicates this motif interacts with a hydrophobic binding groove in subdomain C of the FERM domain. Disorder was observed in the C-terminal basic region of the interacting protein, but coincides with an acidic surface in the FERM subdomain C [11].

It was discovered in the latter study that the N-terminal basic region of the interacting protein (present in ICAM 1–3 proteins, CD43, and CD44) must stabilize the binding, although no direct interaction was detected in this study. A different surface of the C subdomain of FERM has the EBP50 (ERM-binding phosphoprotein 50, also known as NHERF) binding to it. There must be differences between the three ERM proteins although similar in structure and function; ezrin is found mainly in the apical side of epithelial cells, moesin in endothelial cells and radixin in hepatocytes [12].

In many cases, the attachment of ERM proteins to the cytoskeleton is strengthened by phosphorylation of the proteins. The paralleled formation of membrane protrusions in Swiss 3 T3 cells was as a result of activation of the small Rho GTPase, RhoA and not Rac or Cdc42 was able to induce phosphorylation of both radixin and moesin. The enhancement of phosphorylation status of moesin on threonine 558 (a residue also phosphorylated by PKCθ) was enhanced and this bolstered the interaction of moesin with the cytoskeleton, and moesin was found localized at the spreading filopodia [13]. Phosphorylation of radixin on threonine 564 at the C-terminal half by Rho-kinase had no effect on the C-ERMAD to bind F-actin, but attenuated the ability of the C-ERMAD to bind N-ERMAD implying that the activated state of ERM proteins during which the intramolecular interaction between the N- and C- terminal domains is inhibited, can be sustained by the phosphorylation of threonine 564 in radixin, threonines 558 and 567 in moesin and ezrin respectively [14].

ERM proteins can also be phosphorylated by receptor tyrosine kinases. Epidermal growth factor (EGF) receptor can phosphorylate ezrin at tyrosine 145 and 353 (Y145 and Y353). In epithelial kidney cells, Y353 phosphorylation is required for not only the activation of Akt signaling pathway, but also for binding of phosphatidylinositol 3-kinase to ezrin. Likewise, increased phosphorylation of ezrin at the same tyrosine residues was as a result of stimulation of ezrin-transfected LLC-PK1 cells with hepatocyte growth factor (HGF) and this not only upgraded cell migration, but also enhanced intracellular signal transduction. Ezrin Y145 phosphorylation was demonstrated in Jurkat T-cells expressing Lck (a Src family kinase), but not in Lck-deficient cells [10].

There is good documentation of the role of sphingolipids in activating ERM proteins. For example phosphorylation and activation of ezrin in a time- and dose-dependent manner, and in Hela cells, S1P-mediated phosphorylation was found to be through S1P receptor 2 (S1PR2) both endogenously and exogenously as a result of several cell lines such as A549, HEK, MEF, MCF7 and MDA cells, expression of the bioactive sphingolipid, sphingosine-1-phosphate (S1P) [15]. This was required for filopodia formation. In a PKC-dependent manner, S1P stimulation of pulmonary endothelial cells resulted in activation of ezrin and moesin, but not radixin. However, opposite to its known functions, through unclear mechanisms, S1P phosphorylation of ezrin resulted in inhibition of cell invasion, and this could be attributed to the ability of S1P to act on different receptors [16]. In Hela cells, generation of plasma membrane ceramide through breakdown of sphingomyelin by the action of sphingomyelinase precipitated in dephosphorylation of ERM proteins, while ERM proteins hyper-phosphorylation was as a result of decreasing plasma membrane levels of the sphingolipid [17].

Dephosphorylation and inactivation of the proteins through the activities of phosphatases, and via PIP2 hydrolysis orchestrates regulation f ERM proteins. In Hela cells, ceramide drives ERM dephosphorylation through activation of protein phosphatase 1α (PP1α) with the resultant effect of inactivating ERM and subsequent dissociation from the plasma membrane [18]. Correspondingly, overexpression of the small protein tyrosine phosphatase, phosphatase of

regenerating liver-3 (PRL-3) in HCT116 colon cancer cell line resulted in dephosphorylation of ezrin. Through dephosphorylation of threonine 558 moesin can be downregulated by myosin light chain phosphatase. Although, in phorbol 12-myristate 13 acetate (PMA)-stimulated leucocytes, ezrin is inactivated through calpain-mediated cleavage, moesin and radixin are insensitive to fractionation by calpain suggesting that distinct regulatory mechanisms exist for each protein in the same cell [19, 20]. Activated ERM proteins through the FERM domain interact with several proteins in the plasma. In a manner dependent on PIP2, ERM proteins can associate with the cytoplasmic tails of intracellular adhesion molecules -1, -3 (ICAM-1 and -3) and -2 (ICAM-2), as well as the hyaluronic receptor CD44 and CD43 [21, 22].

Through other anchoring proteins like NHERF1 also known as ERM-binding phosphoprotein 50 (EBP50) and NHERF2, ERMs are also known to bind PDZ postsynaptic density protein (PDZ)-containing proteins such as transporters and ion channels. The ERM proteins also associate with membrane glycoproteins such as P-selectin glycoprotein ligand-1 which tether white blood cells to injured tissues [23]. The α-helical domain on the central portion of ERMs can also bind regulatory subunits RII of protein kinase A as well as subunits of HOPS complex (homotypic fusion and protein sorting). Binding of ERM proteins to PKA tethers it to downstream targets to effectcAMP-mediated biological processes such as cell differentiation, proliferation, metabolism, apoptosis, exocytosis, T cell and B cell activation, muscle contraction. Ezrin was shown to bind and link syndecan-2 to the cortical cytoskeleton in COS-1 cells. Unlike other membrane proteins that bind all ERM proteins, the death receptor Fas/CD95 did not bind to moesin but only to ezrin in T lymphocytes [24].

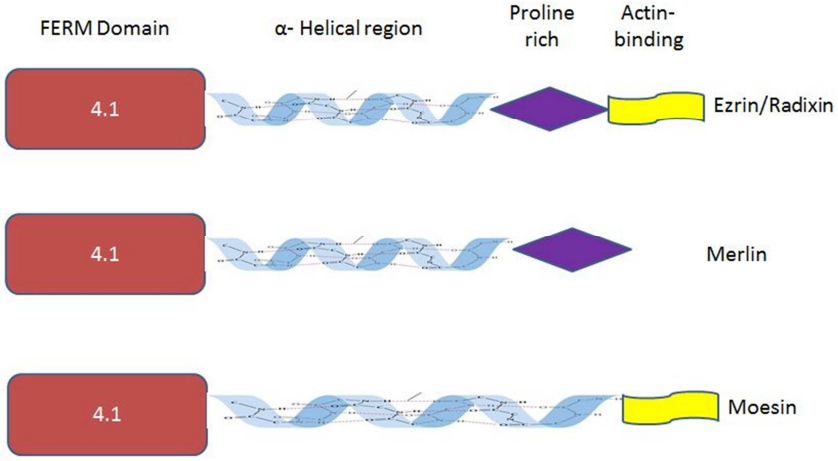


Figure 1. *Structure of the ERM family showing the various regions.*

2. Interface of the FERM/Tail Locusand Reactivity

There are three regions of tight contact described here in addition to several interaction regions of lower complementarity where pockets of water molecules mediate the interaction. The first is the interaction between strand 1 of the tail and lobe F3, where, in addition to the main chain hydrogen bonding that extends the β sheet, the side chain of Leu494 is buried in a hydrophobic pocket formed by residues Ile245, Ile248, and His288 [25, 26]. Interestingly, the position of this tail domain strand matches well with that observed for peptides bound to PTB domains, with the Leu494 bound in a pocket equivalent to that occupied by a hydrophobic residue found upstream of the phosphotyrosine in PTB substrates [27]. The second region of tight contact is between the hydrophobic residues on helices B and D of F2 and hydrophobic face of tail helix A. The third interaction involves the binding of the C-terminal helix D of the tail in a groove on the surface between the two sheets of the F3 β sandwich. In a hydrophobic pocket formed by residues Leu216, Ile227, Lys237, Ile238, and Phe267 the side chains of Phe574 and Met577 bind, and the terminal carboxylate group hydrogen bonds with residues Asn210 and Ser214 [26, 28]. The observation that the FERM/tail interaction is largely disrupted in ERM mutants truncated at residue 575 is explained by the burial of Met577 at the interface. This binding site is analogous to the sites of inositol phosphate binding to PH domains, with respect to its position on F3. In addition, a positively charged loop between strands 1 and 2 of F3 participates in the binding as seen in the PH domains [29]. Interestingly, moesin is the first structural example of a PH/PTB/EVH1 fold that has a substrate (the C-terminal tail domain) bound at both the canonical PTB and PH binding sites simultaneously, underscoring the flexibility of this fold as a ligand binding module and confirming the expectation of Forman-Kay and Pawson [30].

With reference to electrostatics, the interface includes a region of negative charge on the surfaces of FERM lobes F1 and F2 that packs against an area of positive charge on the surface of the tailas shown in Figure 2. Furthermore, lobe F3 contains a region of positive charge noted above that interacts with the largely neutral helix D of the tail. The exposed surfaces of both the domains are dominated by regions of positive charge aside from the FERM/tail-interface [31].

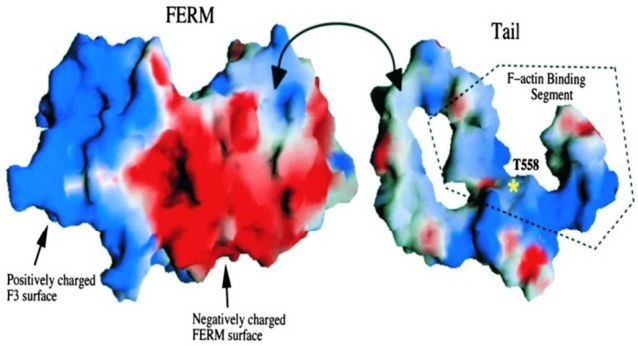


Figure 2. *Structure showing the reaction sites and mechanism of the FERM interface and tail locus (Adapted from: Matthew A. Pearson, Cell, 2000).*

The molecular surfaces of the domains reveal the electrostatic potential (from negative to positive) at the interface. Outlined is the region containing the C-terminal F-actin binding site and the position of Thr558 is marked with an asterisk. In Figure 4C and Figure 5 molecular surfaces and the electrostatic potential were calculated using GRASP. Not visible are the interface surfaces for residues 488–494 of the tail and their docking site.

2.1. Activationof Conformational Changes

Weakening the FERM/tail interaction so as to unmask the membrane-protein and actin binding sites as in will activate the ERM proteins. The remarkably distributed nature of the tail means that its high affinity derives from the binding affinities of the five parts of the extended structure whose interactions are largely independent of each other: strand 1 and helices A, B, C, and D. The net affinity can be very high because of the chelate effect even if all individual affinities are moderate [32]. The pieces are all connected to one another and the cost of losing overall translational and rotational entropy during binding is only paid once in other words. To provide quantitative insight into activation, biophysical studies of the thermodynamics and dynamics of this novel inhibitory mode will be required. However, qualitatively, one clear advantage this binding mode has over a single globular domain that binds as a unit is that, here, multiple independent signals (interactions) can contribute to achieve differing levels of activation by competing with or weakening different parts of the interaction surface [33, 34].

The activation of ERM proteins is influenced by phosphorylation of a specific C-terminal threonine. This residue in moesin, Thr558, is located on helix C of the tail, at an edge of the interface where it is both in contact with the FERM domain and exposed to solvent. Phosphorylation at this position will weaken the helix C/FERM interaction due to both electrostatic and steric effects as the structure shows [35]. With reference to electrostatics, Thr558 is in a positively charged surface, and is positioned opposite to the heart of a negatively charged surface of the FERM domain, where the introduction of the negatively charged phosphoryl group would have a strong detrimental effect. Electrostatic change is important as indicated by a mutation of Thr558 to Asp that mimics this charge change is weakly activating. The side chains surrounding Thr558 approach it closely enough that there is not sufficient room for a phosphoryl group in terms of sterics, so that some structural rearrangement must occur, and, given the proximity of these residues to the interface, any distresses will clearly affect the stability of the complex [36].

It is less clear what the contribution of anionic phospholipid vesicles are to activation, but they bind to both the intact dormant protein and to the isolated FERM domain of ERM proteins. We speculate that the negatively charged phospholipids would have the highest affinity for this part of the interface, and might compete with and weaken the binding of helix D to the FERM domain, given the highly positively charged surface of lobe F3 [13].

2.2. Activated ERM Molecules Potential Binding Sites

Mapped to its last 34 residues is the portion of the tail responsible for F-actin binding, which form tail domain helices B, C, and D in the complex. A simple direct mechanism for the masking of the actin binding site is provided by the intimate involvement of these residues in the FERM/tail interface. The structure seen here does not provide a model for the conformation that these residues will adopt when they bind to actin as suggested by three arguments [37].

Phosphorylation of Thr558 does not block actin binding, but, as discussed above, it must cause at least some rearrangement of the tail.

This region of the tail makes so few intramolecular tertiary interactions that its conformation is clearly heavily dictated by docking onto the FERM domain.

The last 26 residues of the tail is the only part of the domain which has high sequence conservation of residues not buried in the FERM/tail interface. This would only be expected if actin binding places additional constraints on the evolution of these residues, and suggests that these “back-side” residues are more involved in actin binding than they are in the FERM/tail interaction.

There is expectation that the FERM domain has multiple binding sites as a result of an observation that the FERM domain of ERM proteins binds to several membrane proteins and to phospholipids [31].

Considerations of residue conservation and of homology provide guides for future studies aimed at dissecting these binding functions although no experimental data exist to specifically locate these binding sites on the FERM structure. The first guide is the high conservation of surface residues not involved in the interface, as these residues are likely to have a functional importance [38]. The conservation of non-interface residues in the last 26 residues of the tail discussed above would have provided an accurate indication of the location of an interaction site even in the absence of biochemical studies as an example [39]. Strong conservation implicates two patches on the backside of the ERM FERM domain, a large one on lobe F1 and a smaller one at the edge of lobe F2. The second guide is the similarity of lobes F1, F2, and especially F3 to known protein modules. If the binding

sites on these analogous represent true homologies then positions of binding sites may be conserved despite the high degree of sequence divergence and for this reason they need to be documented. The five such sites that can be mapped onto FERM domain. Among these, sites 1, 4, and 5 seem most worthy of attention because site 1 corresponds to the large conserved patch on domain F1, and sites 4 and 5 are both making direct interactions with the tail and are thus masked in the complex [40].

2.3. Interaction of the FERM/Tail in the Tumor Suppressor Merlin

The ERM homolog merlin exists as two alternatively spliced isoforms each containing an N-terminal domain that shares ∼60% sequence identity with the FERM of the ERMs. In dissimilarity, the C-terminal 100 amino acids of merlin isoform I have only ∼20% identity with the ERM tail, while isoform II has a truncated C terminus [39]. The enhanced conservation of residues that lie on the moesin FERM/tail interface, providing strong evidence that the interaction in merlin is equivalent is revealed by the present structural data. A remarkable 81% of the residues that are invariant between the ERM tails and merlin lie on the interface. The poor sequence conservation among the last 34 residues between ERMs and merlin (except for those involved in the interface) interestingly suggests that the FERM/tail interaction is the only common functional constraint on this region of merlin, and is consistent with the lack of a C-terminal F-actin binding site in merlin [41].

These observations confirm that the moesin structure is a good model for merlin isoform I, providing a framework for understanding the mutations that lead to nonfunctional merlin and hence tumor formation. 14 single site substitution mutations that are associated with human tumors. Where they are likely to cause destabilization or mis-folding, six of these residues are buried in FERM domain [27, 42]. Fascinatingly, these buried mutations are at positions throughout the FERM domain (three in F1, one in F2, one in the linker between F2 and F3, and one in F3), indicating that the whole FERM unit is important for merlin function. Of the eight other mutations, three (merlin Leu535Pro, Gln538Pro, and Leu539His) relate to tail residues in moesin (Val518, His521, and Leu522) that are on the interface, at the site of tight association with lobe F2. The remaining five surface mutations are scattered over the FERM domain (two in F1, one in the linker between F1 and F2, one in F2, and one in F3) and do not cluster into a region on the surface [43]. Furthermore, these natural mutations, mutagenesis experiments in Drosophila have been used to identify important functional regions of merlin, including the "blue box" region that includes residues corresponding to 161–167 of the moesin FERM domain. Merlin with lowered activity, similar to that of an isolated merlin FERM domain resulted from a missense mutation (Met177Ile in Drosophila merlin) corresponding to Gln167 in moesin. The neighboring residues 161–163 are on the FERM/tail interface, suggesting that the blue box mutation might lead to weakening of the complex. While moesin Gln167 is not on the FERM/tail interface. In contrast to the missense mutation, deletion of residues in the blue box lead to nonfunctional merlin, which again indicates the importance of proper folding of the FERM domain [44].

2.4. FERM Domainof Other Members of the Band 4.1 Superfamily

The structure of moesin is a useful prototype for the FERM domains of other proteins as sequence conservation indicates. In fact, the six best conserved FERM residues are buried in the structure, consistent with the conservation of the fold throughout the band 4.1 superfamily. Two observations suggest that the FERM domain functions as a single unit rather than a collection of three separate modules although there are three structural domains making up the FERM domain [45]. First, the linkers between the three lobes are rather short (13 and 8 residues), and their sequences are well-conserved in the ERM proteins, merlin, and band 4.1. Second, interacting side chains in this central region, including those from the linkers and the domains, are very well conserved, and two of the aforementioned six highly conserved FERM residues, Gln105 and Gly202, are in this core. Structural changes in one domain would impact and possibly destabilize the other domains as such inter-domain interactions indicate. This conserved central core of residues provides evidence that the FERM domain remains structurally rigid upon activation, leading to the important conclusion that the structure solved here is not only a model for the dormant ERM proteins, but a model for all FERM domains, including those in activated ERMs [46].

The poor sequence conservation of residues on the FERM/tail interface rules out this type of interaction in other FERM domain–containing proteins in contrast to the residues internal to the FERM domain. Nonetheless, the binding sites of other FERM domains may also be regulated by peptide inhibitors, albeit through a set of different specific interactions [47]. Indeed, the highly efficient and versatile inhibition mode revealed here provides a paradigm for thinking of other cytoskeletal proteins that are regulated by masking. Additionally, the structure of the moesin FERM/tail complex presented here opens the door for refined mutagenesis experiments aimed at expounding details of the structure–function relations of ERM proteins, merlin, and all FERM domains [48].

3. Structure

A lot is discovered concerning the intramolecular head – tail association and its effects on protein function through ERM protein structure analysis. Low-angle rotary shadowing electron microscopy shows that radixin point mutants could adopt dramatically different conformation. The amino terminus of ERM proteins is an approximately 1-296 amino acid FERM domain also known as N-terminal ERM interaction domain (N-ERMAD) through which they relate with cell membranes [49]. Through X-ray crystallography the FERM domain has been shown to consists of F1, F2 and F3,

also respectively referred to as A, B and C subdomains that fold and joined together to form an cloverleaf structure, and these subdomains are homologous to ubiquitin, acyl-CoA binding protein and plekstrin homology domains respectively (Figure 5). A central (approximately 200 amino acid) α–helical domain that form coiled coils and mediate interaction with protein kinase A (PKA) closely flanked the FERM region. The carboxylic terminal tail consists of 107 residues, and this terminus contains the F-actin binding site through which ERMs interact with the actin cytoskeleton [50, 51].

Distinct domains within the N-terminal head and C-terminal tail known as N- and C-ezrin-radixin-moesin association domains (N-ERMAD and C-ERMAD respectively) intermediate homotypic and heterotypic head-to-tail interaction in all ERM family members (Figures. 3, 4, 6 and 7). The N-ERMAD is an unstable domain that is inactivated by chemical agents such as sodium dodecyl sulfate (SDS) treatment, and its activity is negatively affected by freeze thawing. C-ERMAD on the other hand is unaffected by chemical treatment [52].

ERMs exist in a dormant, inactive closed conformation within the cytosol in which the C-ERMAD stretches from the F-actin binding site through F2 and F3 to part of the FERM region, thereby concealing both the F-actin and the membrane binding sites from other binding partners [5]. The C-ERMAD covering the FERM is reinforced by the central α-helical domain in that it binds the FERM domain to facilitate masking of both domains. Opening up the binding sites in the FERM domain and those of the F-actin biding sites in the C-terminal domain requires activationof ERMs. Phosphatidylinositol 4, 5-bisphosphate (PIP2)-mediated uncoupling of the C-terminal domain from the FERM domain will achieve this [53].

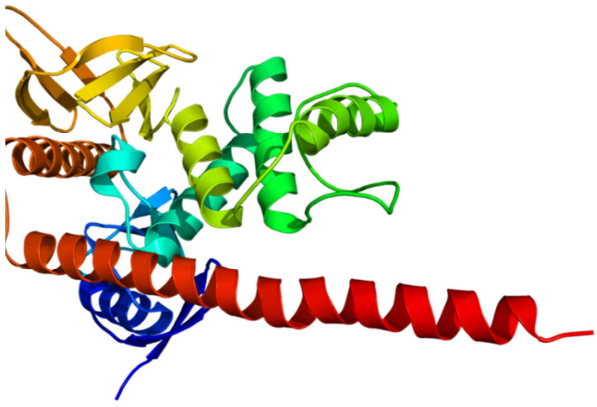


Figure 3. *Structural module of the FERM domain.*

(Adapted from Uniprot)

Forming a compact clover-shaped structure together, the FERM domain is composed of three structural modules (F1, F2, and F3), shown in F3.

F1 (residues 4–82) contains a 5-stranded mixed β sheet packed against an α helix with a short 310 helix prior to the start of strand 3.

F2 (residues 96–195) is composed of five α helices, with an excursion of 36 residues between helices B and C that contains a long loop and a short α helix.

F3 (residues 204–297) consists of a sandwich of two orthogonal antiparallel β sheets followed by a long helix, with a turn of 310 helix in the loop connecting the two sheets.

The center of the clover is filled largely by the 13 residue linker between F1 and F2 (which includes a short α helix) and the 8 residue linker between F2 and F3. The structural predictions of Turunen et al. 1998 were soundly accurate with the three-lobed nature and secondary structure of the moesin FERM domain indicate that, but contradict the prediction made by hydrophobic cluster analysis that the FERM domain consists of a duplication of two 140 residue domains [54].

The structures of proteins whose sequences are not recognizably similar to FERM domains are surprisingly similar to the structure of each of the three FERM lobes. The structure of ubiquitin, a fold that is found in several proteins of dissimilar sequence and function is very similar to F1. The structure of acyl-CoA binding protein, which binds acyl-CoAs of various acyl chain lengths is very similar to F2 [55]. The fold of an adaptable ligand binding module seen for phosphotyrosine binding (PTB), pleckstrin homology (PH), and Enabled/VASP Homology 1 (EVH1) domains is shared by F3. Often present in cell signaling and cytoskeletal proteins, these domains bind peptide and/or phospholipid ligands. Integrated into the FERM domain, the unexpected presence of a PH/PTB-like domain is reminiscent of an unrecognized SH2 domain that combines with other domains to form an amino-terminal module of the Cbl adaptor protein, and serves as a reminder that even well worked out sequence fingerprints are not powerful enough to recognize all homologs [56].

The C-terminal tail adopts an extended, meandering structure that efficiently blocks a large area of the FERM surface, in contrast with the expected globularity of an~80 residue domain. For a polypeptide this large to bind to another protein in such an extended manner is highly unusual. Forming an antiparallel β strand alongside strand 5 of F3, the first ordered residues (488–494) of the tail extend the second β sheet of the F3 module [57]. Residues 495–501 form a poorly ordered connection to residues 502–577 that fold into four major α helices and two short helices that extend across the surface of domains F2 and F3. The structure the tail domain adopts in complex with the FERM domain is unlikely to be stably adopted by an isolated tail domain because the tail domain makes few internal tertiary interactions [58]. In support of this estimation, the free tail is very sensitive while the FERM domain and the FERM/tail complex are relatively stable to proteolysis in crude bacterial extracts. Overall, the tail/FERM interactions bury a remarkable 2950 Å2 (36%) of the tail surface and 2700 Å2 of the FERM surface. The almost perfect sequence conservation among interface residues affords a strong argument that this structure represents the relevant domain complex present in the dormant ERM molecules [59].

3.1. Ezrin

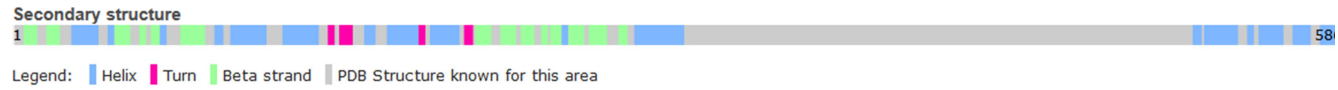
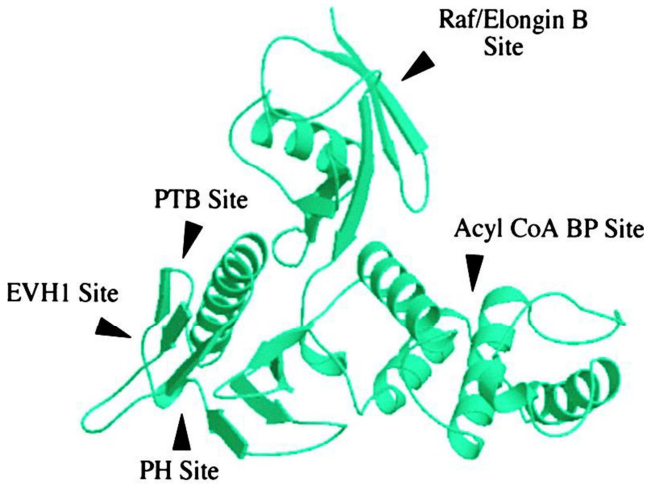


Figure 4. *Annotated secondary structure of Ezrin(Uniprot).*



(Adapted from: Matthew A. Pearson, *Cell*, 2000)

Figure 5. *ERM family protein binding sites.*

The behavior of ezrin and ezrin fragments when expressed in cultured cells first suggested evidence for internal regulation of ERM proteins. The formation of actin-rich micro-spikes is caused by the expression of the C-terminal tail of ezrin in insect cells and mammalian Chinese hamster ovary (CHO) cells, an effect that required the complete actin-binding site in the tail region [60]. Dissimilarly, expression of full-length ezrin had no effect on the actin organization. Expression of the ezrin N-terminal head domain conspicuously did not affect the actin assembly, and when co-expressed with the C-terminal tail, suppressed micro-spike formation. Auto-inhibitory head–tail domain interactions regulated the ability of ezrin to regulate the actin organization as observations suggested. Binding studies with purified accessory proteins offered additional support for negative autoregulation of ERM protein function [61].

The positions of five binding sites on proteins, depicted in Figure 5, that are structurally similar to the three FERM lobes are indicated:

By comparison with the protein interaction site seen for elongin B and the Ras binding domain of Raf kinase;

By comparison with the lipid binding site of the acyl CoA binding protein, but this site is blocked by the loop connecting the F2 helices C and D;

By comparison with the proline-containing peptide binding site on EVH1 domains;

By comparison with the canonical peptide binding site of PTB domains;

By comparison with the inositol phosphate binding sites on PH domains.

3.2. Radixin

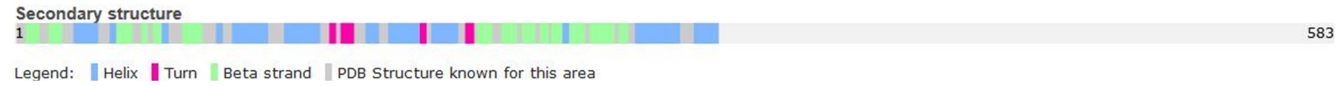


Figure 6. *Annotated secondary structure of Radixin (Uniprot).*

It is suggested that phospholipids could prevent a head–tail interaction by structural analysis of the radixin N-terminus with and without bound inositol-3 phosphate. Protein binding can also facilitate release of the head–tail interaction in ERM proteins, and thus regulate ERM protein activity. For example, Guanosine triphosphate-bound Gα13 binding to radixin also induces a conformational change that facilitated the actin binding, while calcium-dependent binding of S100P to ezrin releases the head–tail interaction and promotes F-actin binding [62].

The some single point mutants extend radixin conformation to 22–25 nm; in contrast, the recombinant wild type radixin has a 12–14 nm globular conformation. An interface with a large surface area with the potential mask ligand-binding sites while maintaining the globular conformation observed in the rotary shadowing EM analysis was showed by a crystal structure of the complex between moesin head and tail domain fragments [63].

3.3. Moesin

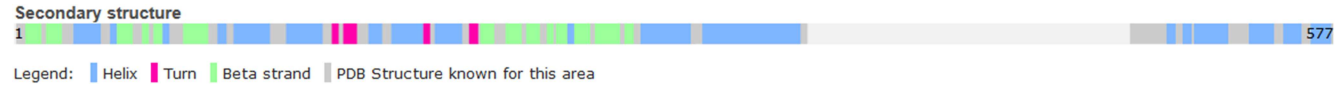


Figure 7. *Annotated secondary structure of Moesin(Uniprot).*

The human moesin FERM (residues 1–297) and tail (residues 467–577) domains were each expressed as selenomethionine (Se-Met) derivatives, and the complex was formed in vitro and crystallized [28]. Crystals of the complex diffracted X-rays to 1.9 Å resolution, and the structure was solved by multiple-wavelength anomalous diffraction (MAD). In the crystal, the moesin FERM/tail complex is present as a domain swapped dimer of complexes with

residues 502–577 of the tail binding to one FERM domain, and residues 488–494 crossing over to interact with a second FERM domain. Because the protein exists in solution as a 1:1 complex, the observed arrangement is a crystallization artifact, and here we describe the structure derived by mapping the observed interactions onto a single complex [64].

4. Functions

The ERM proteins function is regulated by a two-step process of open (active) and closed (inactive) conformation. Resulting in activation of the proteins, they are mainly regulated through conformational changes induced by phospholipids and kinases-mediated phosphorylation [65]. A conserved regulatory threonine phosphorylation residue (T567, T564 and T558 in ezrin, radixin and moesin respectively) located in the C-ERMAD domain is exposed by the enrolment of ERMs to areas of the plasma membrane with increased amount of phosphoinositides such as PIP2 and this effectuates a successive activation mechanism whereby PIP2 first bind to a subdomain in the N-terminal FERM domain followed by plasma membrane translocation and phosphorylation of the threonine residues [66]. Mutation on any of the three lysine-rich consensus sites known to bind phosphoinositides on the FERM domain of ERM proteins inhibits PIP2-ERM interaction and translocation to the plasma membrane. In the phosphorylation of these proteins by other kinases and also in the formation of microvilli PIP2-mediated recruitment of ERMs to the plasma membrane is adequate [10].

Rho-associated protein kinase (ROCK), lymphocyte-oriented kinase (LOK), myotonic dystrophy kinase-related Cdc42-binding kinase, Nck interacting kinase, protein kinase C (PKCα, PKCβ), G-protein coupled receptor kinase 2, NFκB-inducing kinase (NIK) are different signaling protein kinases that can generate phosphorylation of the conserved threonine residue thereby creating stearic hindrance that keeps the FERM and C-ERMAD domains apart, and this stabilizes the active state of ERM proteins in their open conformation [67]. Ezrin can be phosphorylated by cyclic-dependent kinase 5 (CDK5) on threonine 235 which lies between the FERM and C-ERMAD domains, and mutation of this site facilitated ezrin localization to plasma membrane. Loss of cell-cell adhesion and deregulation of cell-matrix interaction is brought about by cancer cell migration is a coordinated process involving different steps. Different factors such as localization of ERMs within the cell, their level of phosphorylation as well as expression profile are responsible for ERM proteins-mediated promotion of tumorigenesis as outlined in several reports [68, 69].

4.1. Ezrin

The membrane-cytoskeleton linker ezrin is mainly expressed in epithelial cells where it associates to the apical actin-rich structures such as microvilli. Latest genetic analyses showed that ezrin is essential for the morphogenesis of epithelial cells. Morphological defects in the apical domain of intestinal and retinal pigment epithelial cells have been observed in ezrin−/− mice. Ezrin knockdown impairs the formation of canalicular apical membrane, resulting in severe achlorhydria in parietal cells [70].

A conserved globular N-terminal domain, called the FERM domain (Four point one ezrin, radixin, moesin), involved in the binding to both phosphatidylinositol 4, 5 bisphosphate and plasma membrane proteins and a C-terminal F-actin–binding domain that resides in the last 34 amino acids is encompassed by ERM (ezrin, radixin, moesin) proteins [61]. Because of an intramolecular interaction between the N-terminal domain and the last 100 amino acids called N- and C-ERMAD (ERM association domain), respectively, in the cytoplasm, ERM proteins exist in a closed conformation. This intramolecular association masks the binding sites for plasma membrane proteins and F-actin. An activation step is required to disrupt this association that occurs through conformational changes induced by sequential binding to PIP2 and phosphorylation of a conserved C-terminal threonine residue [71].

It has been suggested that there is a link between activation of ERM proteins and the signaling pathways triggered by the small GTPases of the Rho family. The ability of ERM proteins to bind the cytoplasmic domain of CD44 is increased by activation of Rho. ERM proteins are required for the formation of focal adhesions and actin stress fibers in response to active RhoA and Rac in permeabilized fibroblasts [72]. The elevation of PIP2 and induction of microvilli with a concomitant recruitment of activated ERM proteins is as a result of A RhoA-dependent activation mechanism for ERM proteins has been proposed on the basis that overexpression of either RhoA or its direct effector, the phosphatidylinositol 4-phosphate 5-kinase. Conversely, ERM protein inactivation with a concomitant microvillus breakdown is as a result of Rac1 activation in T-lymphocytes by chemokine or after TCR engagement. These observations suggest that Rho GTPases can function as upstream regulators of ERM proteins [73, 74].

However, ERM proteins can act as upstream regulators of Rho GTPases by binding to the Rho GDP dissociation inhibitor (RhoGDI) as in vitro and in vivo studies have indicated. This association is thought to displace RhoGDI from Rho GTPases, allowing them to be activated by their specific guanine nucleotide exchange factors. As has been reported, there is an association of ERM proteins with the exchange factor Dbl in vitro as well as in vivo [75]. An active form of ezrin has been shown to activate the small GTPase Rac1 with a concomitant disassembly of adherens junctions in epithelial cells. Drosophila moesin negatively regulates the Rho1 pathway as suggested in a genetic analysis in Drosophila. Therefore, supporting the idea that ERM proteins function both upstream and downstream of Rho GTPases, these data reveal a complex relationship between ERM proteins and the small GTPases [76].

A novel GEF that interacts with ezrin and that activates the small GTPaseRhoG using a yeast two-hybrid screen has been

identified. RhoG is believed to function upstream of Rac1 and Cdc42 and shares significant homology with Rac1 (72% identity). Nevertheless, RhoG signals in parallel of Rac1 and Cdc42 rather than upstream as suggested by other studies. Although several regulators of the RhoA, Rac1 and Cdc42 GTPases have been characterized few regulators of RhoG have been identified consequently resulting in a controlled process by activated RhoG are poorly understood [77]. Trio, through its N-terminal DH/PH tandem, functions as a RhoG exchange factor and activation of RhoG by TrioGEF1 regulates neurite outgrowth. Macropinocytosis in fibroblasts is stimulated by SGEF, another exchange factor for RhoG. ELMO has been identified as a specific effector of RhoG by recent studies. In cell of apoptotic cells, and in RhoG-mediated neurite outgrowth, ELMO has been implicated. ELMO cooperates with Dock180 to promote downstream Rac activation in these processes. Moreover, it has recently been reported that an interaction between ERM proteins and ELMO [78].

The interaction between ezrin and a novel GEF, PLEKHG6 (pleckstrin homology domain containing family G with RhoGef domain member 6) is characterized here. PLEKHG6 displays an exchange activity toward RhoG and to a much lesser extent toward Rac1. We show that ezrin recruits PLEKHG6 to the apical surface of epithelial cells where it promotes the activation of RhoG [61]. Ezrin also forms a ternary complex with PLEKHG6 and the RhoG effector ELMO, indicating that ezrin interacts with upstream and downstream regulators of RhoG in addition to its interaction with PLEKHG6 and RhoG. We establish that the interaction of ezrin with PLEKHG6 is critical for PLEKHG6-induced morphological changes at the apical surface of epithelial cells but is not necessary for its catalytic activity. Furthermore both ezrin and PLEKHG6 are required for macropinocytosis in epidermal growth factor (EGF)-stimulated A431 cells as is shown [45].

Aberrant intracellular signal transduction triggered by growth factors is as a result of abnormal localization of ERM proteins which is a leading factor contributing to this result. For example, in breast carcinoma, ezrin which was originally situated at apical structures in normal cell was found translocated to the cytoplasm and plasma membrane and this aberrant localization led to the acquisition of an epithelial-mesenchymal transition (EMT) in which cells loss their normal differentiated, planar and apical-based polarity and anchorage dependent architecture and instead acquire metastatic phenotype that correlated with poor prognosis [79]. Recruitment and activation of Fes kinase at the cell membrane is caused by interaction of ezrin with Fes kinase in an assortment of cells where it facilitates HGF-mediated loss of cell-cell and cell-ECM contacts resulting in cell migration as revealed by wound healing assay. In this interaction, ezrin not only promoted the formation of membrane protrusions but also localized to the leading edge of migrating epithelial cells [80].

Moreover, a higher number of abnormally long microvilli that are no longer restricted to the apical pole are a result of a constitutively active Ezrin. Normal cell adhesion and polarization are affected when microvilli are no longer restricted. This indicates a potential developmental role for Ezrin, where it diverts the highly dynamic microvilli away from cell–cell contact sites, so that mature junctions can form in a stable manner [68]. ERMs can act as downstream effector of PKC to mediate cell migration when the latter was gingered with phorbol-ester correspondingly and upon phosphorylation of the ERM proteins by PKCα. A switch in phosphorylation site of the transmembrane receptor CD44 from Ser325 to Ser29 is caused by PKC activation by phorbol-ester and this phosphorylation regulated the association of ezrin with CD44 to promote directional cell movement triggered by CD44 [81]. Ezrin binds cell-neural adhesion molecule (L1-CAM) to advance progression of colorectal cancer in that RNA interference of ezrin activity inhibited tumor metastasis mediated by L1-CAM. The metastatic and invasive capabilities of MDA-MB-231 and MCF10A breast cancer cell lines were reduced by the agitation of ezrin activity with small hairpin RNA technology in 3D matrigel matrix. Cell spreading resulted from ezrin Y145 phosphorylation in mouse mammary carcinoma cell line SP1 and in pig kidney epithelial LLC-PK1 cells [82]. Heightened manifestation of ezrin has been reported in LTE, BE1, H446 and H460 lung cancer cell lines, and a considerable reduction in migration, multiplication and invasion was observed upon siRNA-mediated knockdown of ezrin. Overexpression of ezrin has been reported in high grade prostate cancers and this was attributed to increased expression of oncogenic c-Myc. Interestingly, ezrin itself through a feedback loop involving the Akt/PI3K pathway can control c-Myc levels and this is crucial for cell migration and invasion. Ezrin overexpression has been shown in other cancer such as pancreatic carcinoma, rhabdomyosarcoma and osteosarcoma [83].

4.2. Radixin

Unlike ezrin, however, radixin has been implicated in prostate cancer progression even though much is not known about the function of radixin in cancer; and impairment of radixin in human pancreatic cancer cell line by shRNA not only significantly diminished cell proliferation, survival, adhesion and invasion but also enhanced expression levels of the cell-cell adhesion molecule, E-cadherin [84]. Constitutive opening of the membrane and F-actin binding domains is as a result of phosphorylation of a conserved threonine 564 residue which is sufficient to prevent the interaction of the FERM domain at the N-terminus with the F-actin binding domain at the C-ERMAD terminus in radixin. In a manner dependent on Vav (a guanine exchange nucleotide factor for Rac1) activity, downregulation of radixin levels resulted in an increase in Rac1 activity [85]. Indeed, in Madin-Darby canine kidney (MDCK) epithelial cells, phosphorylation of radixin on this site (T564) by the G protein-coupled receptor kinase 2 (GRK2) was able to initiate membrane protrusions as well as elevated locomotion of the cells as determined by wound healing assay [86]. A novel function in which the protein appeared to display metastasis has been reported dissimilarly to the above-mentioned positive roles of radixin

in tumorigenesis. High increase in cells spreading enhanced cell-cell adhesion and acquisition of epithelial phenotype caused by agitation of radixin activity in the metastatic prostate cancer cell line PC3 by siRNA technology according to this report [64, 87].

4.3. Moesin

The stable Glu-MT levels are negatively regulated by moesin and ezrin, two members of the ERM family of cytoskeletal regulatory proteins. They also inhibit retroviral infection as has been demonstrated. A recent study has reported that the moesincontrols stable microtubule formation and inhibits transduction of HIV-1 vectors having VSV-G protein in the rat cells [88]. Results from oyher studies suggested that moesin regulates cytoskeleton rearrangement to suppress HIV-1 replication somewhere after virus entry. Other studies suggest that in the case of R5-tropic virus infection, the moesin-mediated enhancement of infection is dominant in comparison with moesin-mediated suppression of HIV-1 replication, if any, after entry. The EZ-N protein suppressed the X4-tropic HIV-1 infection in TE671/CD4 and 293T/CD4 cells, but did not significantly in HeLa/CD4 cells [76, 89].

Increased tumor size and invasive capability has been correlated with expression of moesin, and there was an aberrant trafficking of the protein from plasma membrane to the cytosol in oral squamous carcinoma cell (OSCC) in which moesin was knocked down [90]. There was no change in expression levels of ezrin and radixin although high grade glioblastoma showed high expression levels of moesin. Moesin promoted tumor cell invasion in that in vitro 3D cell migration assays revealed that moesin depleted-cells exhibited reduced invasiveness [91]. Moesin has been shown to induce EMT in human mammary cell MCF10Aand is considered an important promoter of metastasis, there are now emerging reports that moesin is upregulated in different human cancer cell lines as well as a marker of EMT. In the same vein, high level of moesin was also found in head and neck squamous cell carcinoma. While the level of ezrin expression was unaffected, but its phosphorylation status did change although both moesin and radixin were found upregulated in lymph node metastases of pancreatic cancer [92].

5. Conclusion and Future Perspectives

The ERM proteins play very important functions in mediating signal transduction and maintaining cellular integrity from a variety of extracellular inputs through their interaction with different receptor tyrosine kinases (RTKs) such as EGFR and HGFR, adhesion and adaptor proteins such as E-cadherin, ICAM-1,2,3, NHERF and CD44, and other signaling pathways such as PI3K/Akt, cAMP/PKA and the Rho GTPases, all of which have been implicated in tumorigenesis; thus, making ERM proteins a crucial target in development of novel therapeutics in fighting cancer progression and other related disease conditions where the protein is implicated. Although, several works have been done on the function and structure of this protein, detailed understanding of the mechanisms of their interactions with other proteins as well as their activation is still lacking and requires further investigation.

Abbreviations

EGF: Epidermal growth factor
EMT: Epithelial-mesenchymal transition
ERM: Ezrin, radixin, moesin
FERM: Four point one ERM domain
HGF: Hepatocyte growth factor
ICAM: Intracellular adhesion molecule
LOK: Lymphocyte oriented kinase
NHERF: Na^+-H^+ exchanger regulatory factor
PIP_2: Phosphatidylinositol 4, 5-bisphosphate
PKA/C: Protein kinase A/C
RTKs: Receptor tyrosine kinases
S1P: Sphingosine-1-phosphate

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