



Molecules in focus

Radixin: cytoskeletal adopter and signaling protein

Klaus P. Hoefflich^{a,b}, Mitsuhiro Ikura^{a,b,*}

^a *Division of Molecular and Structural Biology, Ontario Cancer Institute Toronto, Ont., Canada*

^b *Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, Ont., Canada M5G 2M9*

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Abstract

Radixin functions as a membrane–cytoskeletal crosslinkers in actin-rich cell surface structures and is thereby thought to be essential for cortical cytoskeleton organization, cell motility, adhesion and proliferation. This modular polypeptide consists of a long, central helix, termed the α -domain, which connects an N-terminal 4.1/ezrin/radixin/moesin (FERM) domain required for membrane binding and a C-terminal region that contains a major actin-binding motif. Conformational regulation of radixin protein function occurs by association of the FERM and C-terminal domains, whereby the membrane- and actin-binding activities are mutually suppressed and the protein is thought to take an inactive ‘closed’ form. Further analyses of radixin and its family members have also revealed associations with human disease. With the rudimentary state of our present knowledge and the pivotal roles these proteins play, studies on this protein family are sure to continue to attract considerable interest.

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1. Introduction

The cortical cytoskeleton of eukaryotic cells performs many dynamic functions, including transmembrane signaling, growth regulation, differentiation, and the determination of cell shape, adhesion, migration, and division. The ezrin–radixin–moesin (ERM) protein family has been proposed to play structural and regulatory roles in many of these plasma membrane-based processes by functioning as membrane–cytoskeletal crosslinkers in actin-rich cell surface structures (see Bretscher, Edwards, & Fehon,

2002; Tsukita & Yonemura, 1999 for a comprehensive review). In the 1980’s these proteins were first purified and molecularly cloned: radixin was originally identified as a constituent of adherence junctions in rat liver; ezrin was first isolated as a component of intestinal microvilli that is tyrosine-phosphorylated by epidermal growth factor receptor; and moesin was originally identified as a heparin-binding protein abundant in bovine uterus smooth muscle cells. Immunofluorescence experiments in cultured cells confirmed that radixin, ezrin, and moesin proteins are localized at cell surface structures such as apical microvilli, filopodia, uropods, ruffling membranes, retraction fibers, the cleavage furrow of dividing cells, and adhesion sites where actin filaments are associated with the plasma membrane.

In 1993 the positional cloning of the human gene for neurofibromatosis-2 (NF2), a disorder characterized by bilateral schwannomas of the eighth cranial

Abbreviations: CTD, carboxy-terminal domain; ERM, ezrin/radixin/moesin; FERM, 4.1/ezrin/radixin/moesin; NF2, neurofibromatosis-2

* Corresponding author. Tel.: +1-416-946-2025; fax: +1-416-946-2055/6529.

E-mail address: mikura@uhnres.utoronto.ca (M. Ikura).

nerve and other tumors of the central nervous system, led to the identification of a tumor suppressor with significant homology to radixin (reviewed in Reed & Gutmann, 2001). This suggested that the NF2 protein may also act at the membrane/cytoskeletal interface, and accordingly, this protein received the moniker, merlin (moesin, ezrin, radixin-like protein). Homologues of ERM proteins have subsequently also been found in genetically-tractable model organisms, such as *Drosophila melanogaster* and *Caenorhabditis elegans*, although the number of family members is limited to only one in flies and two nearly identical genes in nematodes. While this may suggest a single basic function for ERM proteins, recent genetic studies in mice do not point to complete functional redundancy but rather to overlapping but distinct functions of ERM proteins (Bretscher et al., 2002; S. Tsukita, personal communication). Importantly, an understanding of the structure–function relationships of the ERM family is quickly emerging and there are many open questions that are requisite to grasp exactly what role these molecules play in integrating a large variety of cellular processes.

2. Structure

Significant progress has been made towards understanding both the structure and function of ERM proteins. The genes for the four proteins reside on different human chromosomes: radixin is on chromosome 11 (11 exons); ezrin is on chromosome 6 (13 exons); moesin is on the X chromosome (12 exons), and NF2 is on chromosome 22 (17 exons). Although two merlin isoforms exist as a result of alternative splicing, no evidence of alternative splicing has been reported for the ERM proteins. Radixin (~80 kDa), ezrin (~82 kDa) and moesin (~75 kDa) are very closely related to the band 4.1 superfamily on account of a shared ~300 residue four-point one, ezrin, radixin, moesin (FERM) domain at the amino-terminus (Fig. 1A). FERM domains localize proteins to the plasma membrane and are found in other cytoskeletal proteins, such as erythrocyte band 4.1 and talin, and several tyrosine kinases and phosphatases. The last 34 residues of the ~100 amino acid carboxy-terminal domain (CTD) of radixin consists of a (filamentous) F-actin-binding site which

is notably not conserved in merlin. Spanning these globular domains lies an α -helix-rich domain, termed the α -domain. Recent biophysical studies indicate that the radixin α -domain is an extremely long (240 Å in length from N- to C-terminus), linear monomer with an enhanced number of electrostatic, salt bridge interactions predicted to contribute synergistically to its thermal stability (Hoefflich et al., 2003). Interestingly, the merlin α -domain contains significantly fewer charged amino acids and thereby the propensity for salt bridging would appear to be less. This could suggest different structural and dynamic properties of this central linker domain and may partially explain some of the unique cellular and biochemical features of merlin/NF2 (Reed & Gutmann, 2001).

Several crystal structures for the free, CTD-inhibited and substrate-bound FERM domain of recombinant ERM proteins have been reported (Hamada, Shimizu, Matsui, Tsukita, & Hakoshima, 2000; Hamada et al., 2003; Pearson, Reczek, Bretscher, & Karplus, 2000). The FERM domain is composed of three subdomains with fold homology to ubiquitin, acyl-CoA-binding protein and the pleckstrin-homology domain. The CTD by comparison adopts an extended structure in which the F-actin-binding region is buried in the FERM interface. As attempts to crystallize full-length ERM proteins or the α -domain alone have been unsuccessful until now, a structural model in which a single helix for the α -domain (Hoefflich et al., 2003) is placed spanning the FERM and C-terminal domain structures as determined by X-ray crystallography is depicted in Fig. 1B. Importantly, comparison among the published crystal structures now allows for the definition a structural mechanism of activation. For instance, comparison of FERM domain structures has revealed that binding to the phospholipid bilayer (ascertained by radixin FERM domain complexed with the inositol-(1,4,5)-trisphosphate head group of PIP₂) induces local conformational changes promoting the release of the FERM domain from inhibitory CTD association and markedly stimulates the binding of ERM proteins to adhesion molecules. For example, while moesin does not appreciably bind CD44 in vitro at physiological ionic strength, in the presence of PIP₂ the inhibitory domain is released and the ERM protein binds directly to the cytoplasmic domain of CD44 at relatively high affinity (K_d of moesin is approximately 10 nM) (Hirao et al., 1996). This well documented

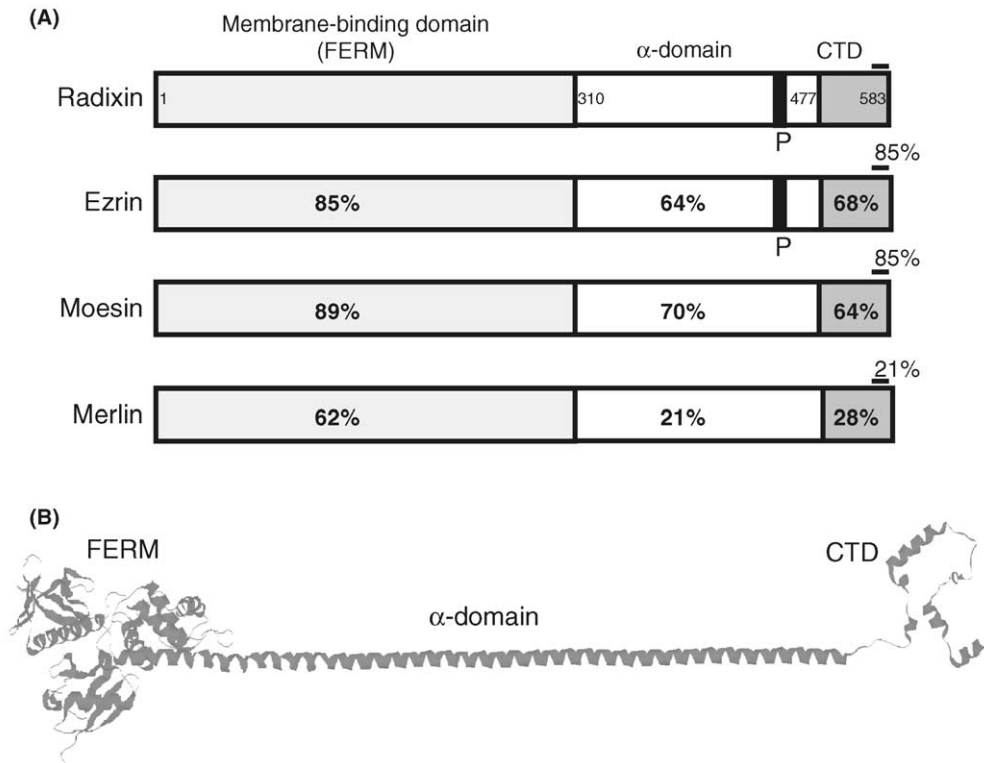


Fig. 1. Schematic representation of the protein structure for radixin, ezrin, moesin, and merlin/NF2. (A) Comparison of the domain organization and sequence identity between murine ERM proteins. NCBI accession numbers are CAA43087, AAA36200, AAA51790, and 620479A, respectively. Residue numbers for radixin are depicted. P, polyproline domain. (B) Model of radixin structure-based on experimentally-derived structural features. FERM and CTD protein database (PDB) files are 1E5W and 1EF1, respectively.

masking model has contributed to this protein family becoming a paradigm for conformational regulation.

3. Biological function

Conformational regulation of radixin function occurs by intramolecular and intermolecular association of the FERM and C-terminal domains, whereby the membrane- and actin-binding activities are mutually suppressed and the protein is thought to take a closed inactive form (Fig. 2). So by what means to the terminal domains dissociate? Evidence suggests that masked ERM molecules are activated by binding phosphatidylinositol 4,5-bisphosphate (PIP₂) to the FERM domain. Subsequent growth factor-induced phosphorylation at C-terminal threonines (Thr-564 in

radixin) by Rho-associated kinase, protein kinase C (PKC)- α or PKC- θ stabilizes unmasked ERM proteins in an open activated form and regulates binding to actin (Bretscher et al., 2002; Tsukita & Yonemura, 1999). These findings are further supported by radixin mutations that mimic Thr-564 phosphorylation, by substitution with glutamic acid, resulting in the formation of persistent actin-based microvillar structures and partial compensation of ERM protein function (Bretscher et al., 2002; Tsukita & Yonemura, 1999). In addition, low-angle rotary-shadowing electron microscopy studies have furthermore suggested that the α -domain also plays a dynamic role in radixin protein activation (Ishikawa et al., 2001).

Activated ezrin, radixin and moesin have been shown to join actin filaments to CD43, CD44, and ICAM1-3 cell adhesion molecules and various

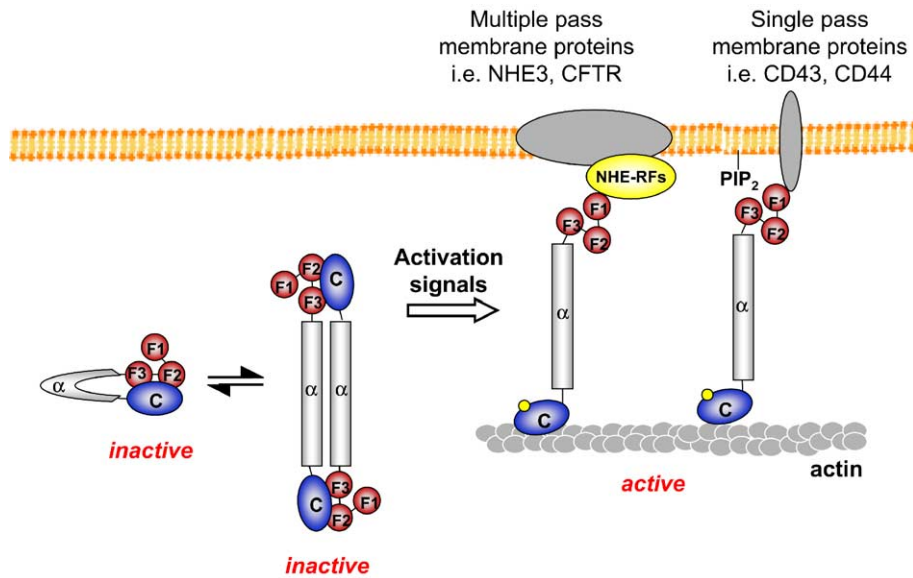


Fig. 2. A working model for the conformational regulation of radixin. Following activation to unmask binding sites, ERM proteins can associate with the membrane and cytoskeletal components. See text for details. F1, four-point one, ezrin, radixin, moesin (FERM) subdomain; α , α -domain; C, carboxy-terminal domain; P, phosphorylation; PIP₂, phosphatidylinositol 3,4-bisphosphate.

membrane channels and receptors, such as the Na⁺/H⁺ exchanger-3 (NHE3), cystic fibrosis transmembrane conductance regulator (CFTR) and the β_2 -adrenergic receptor (Bretscher et al., 2002; Tsukita & Yonemura, 1999). These crosslinking activities have been proposed to lie downstream of signals mediated by Rho GTPase. In *Drosophila* for instance, an ERM protein is required for anchoring microfilaments, maintaining cell shape and coordinating the actin cytoskeleton for proper anteroposterior polarity (Polesello, Delon, Valenti, Ferrer & Payre, 2002). However, although ERM proteins have a crucial role in mediating Rho-induced cytoskeletal rearrangements, recent evidence also places ERM proteins upstream of Rho in a positive feedback loop by sequestering the RhoGDI inhibitor. Further analyses of ERM family members have also revealed associations with human disease. Mice lacking radixin are characterized by a breakdown of hepatocyte apical microvilli which ultimately results in mild liver injury similar to human conjugated hyperbilirubinemia in Dubin–Johnson syndrome (Kikuchi et al., 2002). Hamartin, the protein encoded by the tuberous sclerosis complex-1 (TSC1) tumor-suppressor gene, has also been linked to ERM proteins in regulating cell adhesion and

possibly also hamartoma development (Lamb et al., 2000).

4. Possible medical applications

Although many have speculated that the ERM proteins may play a role in cancer, with the exception of merlin/NF2, data concerning their expression in tumors is rather limited. Radixin and moesin were found to be down-regulated in some lung adenocarcinomas, suggesting that these two molecules may function as tumor suppressors in the early oncogenic stages of lung adenocarcinoma (Tokunou et al., 2000). However, the precise roles of these proteins in cancer cells require further investigation. It may be interesting that the radixin gene is localized in 11q23, one of the chromosomal regions commonly showing loss of heterozygosity in a variety of human tumors, including lung, breast, ovarian, colon, and malignant melanoma (Wilgenbus, Milatovich, Francke, & Furthmayr, 1993).

Importantly though, merlin is a bone fide tumor suppressor for hereditary NF2 and holds promise for medical intervention (Reed & Gutmann, 2001; Sun, Robb,

& Gutmann, 2002). NF2 is an autosomal dominant disease affecting 1 in 35,000 people that predisposes individuals to multiple nervous system tumors, including schwannomas, meningiomas, and ependymomas. In tissue culture assays, inhibition of merlin results in varied actin organization during cell spreading, abnormalities in cell attachment and reduced cell motility, all events known to contribute to the initiation of tumor progression (Bashour, Meng, Ip, MacCollin, & Ratner, 2002; Reed & Gutmann, 2001). Similarly, malignant tumors that arise in mice with a targeted mutation in the NF2 gene have highly motile and metastatic cells (McClatchey et al., 1998). Transfection of NF2 gene suppresses a v-Ha-Ras-induced malignant phenotype and inhibits the growth of NIH 3T3 cells, confirming the role of NF2 as a tumor suppressor. This is an exciting area of research that will contribute to our fundamental understanding of tumorigenesis and potentially the development of potential drug therapies.

5. Perspectives

In summary, as the examples mentioned above illustrate, timely activation of radixin and its ERM family members plays an important role in regulation of the cortical cytoskeleton. While the largest body of work relating to these proteins has focused on conformational regulation as a signal transduction model, recent efforts using genetic models have revealed greater insights into ERM biological function. However, we are only skimming the surface of possible *in vivo* functions, interacting partners and molecular mechanisms in signaling cascades. It is also noteworthy that these proteins are involved in cellular processes that are linked to tumorigenesis. They may, therefore, represent potential drug targets for anticancer therapy, but this notion will require further investigation to clarify.

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