

ERM proteins and NF2 tumor suppressor: the Yin and Yang of cortical actin organization and cell growth signaling

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The ERM (ezrin, radixin and moesin) family of proteins are linkers that tether actin microfilaments to the plasma membrane. Merlin, the *NF2* tumor suppressor gene product, is highly homologous to ERM proteins. In ERM proteins and merlin, interdomain binding promotes auto-inhibition and homo-oligomerization or hetero-oligomerization. Recent studies have revealed that ERM proteins transduce growth signals, and have shed new light on how merlin links cell growth to the cytoskeleton.

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Abbreviations

ERM	the ezrin, radixin and moesin family of proteins
ERMAD	ERM association domain
HGF	hepatocyte growth factor
NF2	neurofibromatosis type 2
PKC α	protein kinase C α
PI 3-k	phosphatidylinositol 3-kinase

Introduction

The ERM (ezrin, radixin and moesin) family of proteins were originally characterized as structural components of the cell cortex. Merlin, also named schwannomin, was identified by genetic approaches aiming to characterize the molecular basis of neurofibromatosis 2 (NF2). This is an autosomal dominantly inherited disorder that predisposes the patient to the development of multiple benign tumors of the central nervous system, such as schwannomas and meningiomas. The *NF2* gene is also implicated in the development of sporadic schwannomas and meningiomas, as well as mesotheliomas induced by asbestos [1]. ERM proteins and merlin display a similar structural organization. They share extensive homology in their amino-terminal domain, which is called the FERM domain. The carboxy-terminal domains of ERM proteins and of merlin are more distantly related. Merlin lacks a conserved actin C-terminal binding site present in ERM proteins [2]. Like ERM proteins, however, merlin contains actin binding sites in its amino-terminal domain [3,4,5*].

These proteins form intramolecular and intermolecular associations between their amino-terminal and carboxy-terminal domains, which are called N-ERM and C-ERM Association Domains (N-ERMADs and C-ERMADs, respectively) [6]. Intramolecular association causes the proteins to adopt closed conformations in which the

membrane and cytoskeleton binding sites are masked. Thus, these proteins need to be conformationally activated to fulfill their functions.

A recent review has described in great detail the properties of ERM proteins and merlin, as well as their partners [6]. We thus focus this review on recent advances in understanding the mechanisms of activation of these proteins and on how these activated proteins transduce growth signals, with a special emphasis on tumor development.

Mechanisms of activation

ERM proteins are maintained in the closed conformation through the strong intramolecular N-/C-ERMAD interactions [6]. The crystal structure of the moesin N-ERMAD–C-ERMAD complex revealed that the C-ERMAD has an elongated structure that masks a large surface of the globular N-ERMAD domain [7**].

In cells, several stimuli are known to activate the ERM proteins. Thrombin activation of platelets induces membrane extensions containing moesin [8]. EGF stimulation of human carcinoma A431 cells induces membrane ruffles containing ezrin [6]. Activation of the Rho pathway stimulates the formation of ERM-mediated microvilli-like structures in fibroblasts [9–11]. All these stimuli induce ERM phosphorylation. Furthermore, epidermal growth factor stimulation of A431 cells induces ERM oligomer formation, suggesting that the intramolecular interaction has been broken to allow intermolecular interaction to occur [6].

The phosphorylation of a conserved threonine residue in the C-ERMAD (Thr567 in ezrin, Thr564 in radixin, Thr558 in moesin) of ERM proteins drastically reduces the N-ERMAD–C-ERMAD interaction *in vitro* [12,13**]. From the structural model of the N-ERMAD–C-ERMAD complex, it can be predicted that the phosphorylated residue destabilizes the interaction through electrostatic and steric effects [7**]. *In vivo*, an ezrin T567D mutant that mimics the phosphorylated form, is morphogenic (Figure 1). Analysis of the ezrin mutants T567D and T567A and the phosphorylated ERM species using a phosphospecific antibody has revealed that the phosphorylation of ERM proteins is a membrane event and that this phosphorylation converts inactive oligomers into active monomers [14**] (Figure 2). The ERM oligomers, the exact configuration of which is not known, might thus represent a transition form in the pathway of activation. The deactivation of ERM linkers is equally important to the dynamics of actin-rich membrane projections, and this might be triggered by dephosphorylation. Indeed, treatment of phosphorylated moesin with purified protein phosphatase 2C inactivates

its binding to actin [15]. *In vivo*, dephosphorylation of ERM proteins correlates with microvilli breakdown induced by anoxia or apoptosis [16,17].

Another factor required for ERM activation is phosphatidylinositol-4,5-bisphosphate (PIP₂), which binds to the FERM domain of ERM proteins. This interaction involves three clusters of lysines that are part of a groove in the FERM domain [18•,19•]. Mutagenesis of these residues abrogates membrane localization of ezrin, indicating that this phospholipid is a major determinant of ERM membrane localisation [18•]. *In vitro*, PIP₂ is required together with phosphorylation to maintain moesin in an active conformation [13•]. *In vivo*, overexpression of phosphatidylinositol 4-phosphate 5-kinase, which increases the level of PIP₂, enhances ERM phosphorylation [11]. PIP₂ binding to the FERM domain of ERM proteins might trigger a conformational change; this has been observed in a co-crystal containing the FERM domain and the inositol 1,4,5-triphosphate (IP₃) [19•]. This active conformation, however, has also been observed in a crystal that does not contain IP₃ or PIP₂ [20•].

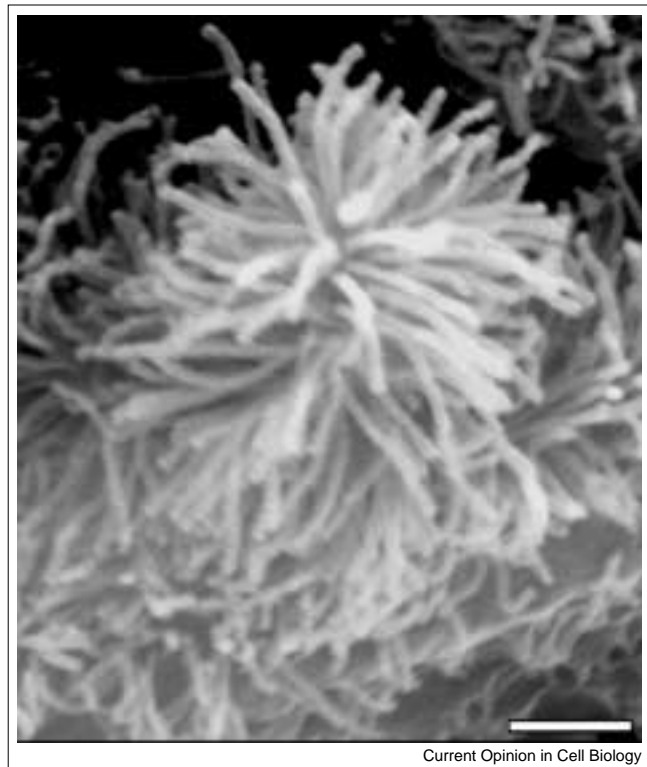
Merlin activation also involves breaking the N-ERMAD–C-ERMAD interaction (at least in isoform 1, which is conserved throughout evolution [21]). Like the ERM proteins, the actin binding site of merlin is masked because of the N-ERMAD–C-ERMAD interaction [2], and this interaction is also regulated through phosphorylation. It is the phosphorylation of Ser516, controlled by the Rac pathway, that inhibits the N-ERMAD–C-ERMAD interaction, however, and not phosphorylation of the conserved threonine residue equivalent to Thr567 in ezrin [22•]. It is not yet known whether PIP₂ binds to and activates merlin. The N-ERMAD–C-ERMAD interaction of merlin is weaker and more dynamic than that of ERM proteins, since the masking of binding sites is not complete and oligomers form spontaneously [23•,24,25•]. In merlin, the active form has not yet been formally identified. The N-ERMAD–C-ERMAD interaction and dephosphorylation seem to be required for it to be active, however, raising the possibility that merlin oligomers might be the active form of this tumor suppressor protein [22•,26•]. Intriguingly, the merlin C-ERMAD has a stronger affinity for ezrin N-ERMAD than for merlin N-ERMAD, and ezrin–merlin hetero-oligomers have been detected [23•,25•]. These observations suggest that ERM proteins might control the activation of merlin or, reciprocally, that merlin might be a regulator of ERM activation.

Signaling functions of ERM proteins and merlin

Activation of ERM proteins and their interaction with both the membrane and actin filaments controls cell morphogenesis, adhesion and motility, and also transduces growth signals for proliferation and survival.

A requirement for ERM proteins in the morphogenesis of specialized domains of the plasma membrane has been

Figure 1

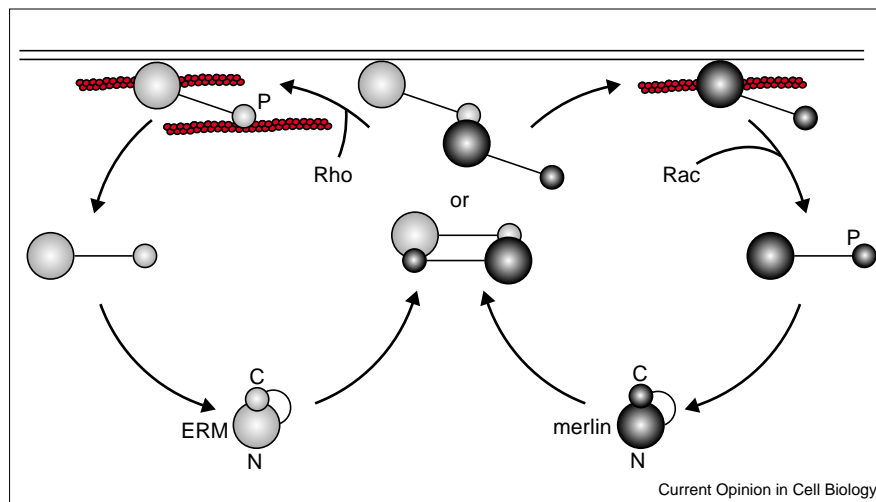


Scanning electron microscopy showing the morphogenic effect of the ezrin T567D mutant in epithelial cells. This active form of ezrin induces tufts of microvilli on the cell surface. Bar: 1 μ m. Reproduced from *The Journal of Cell Biology* [14•] with the permission of the Rockefeller University Press.

reported in several different systems. Ezrin, a protein found primarily in epithelial cells *in vivo*, is involved in the biogenesis of apical microvilli and in the formation of a functional epithelium [27,28•,29,30]. Moesin, which is expressed in lymphoid and endothelial cells, is important for the redistribution of adhesion molecules to the uropods of activated lymphocytes [31]. Microscale chromophore-assisted laser inactivation of radixin and the use of antisense oligonucleotides with moesin and radixin have revealed a role for these proteins in the formation of nerve growth cones [32,33].

ERM proteins regulate cell adhesion through different means. Association of ERM proteins with the cytoplasmic domain of adhesion membrane proteins may serve to recruit these proteins to a specific membrane domain and facilitate adhesion ([6]; see also Update). ERM proteins also control adhesion through the Rho pathway. The relationship between ERM proteins and Rho is complex: as ERM proteins are both upstream and downstream in the Rho activation pathway, they probably form an amplification feedback system [6]. Moesin has been characterized as an essential protein for the formation of focal adhesions in response to active Rho in permeabilized fibroblasts [34].

Figure 2



Model of activation of ERM proteins and merlin. ERM proteins and merlin exist in a closed conformation in the cytoplasm in which their membrane and actin binding sites are masked. Opening of the protein may lead to the formation of homo-oligomers or hetero-oligomers. For ERM proteins, the phosphorylation of a C-terminal residue mediated by the Rho pathway induces the transition, at the membrane, from inactive oligomers to active monomers. For merlin, the phosphorylation of a C-terminal residue mediated by activated Rac inactivates the protein. Phosphorylated merlin does not bind to the actin cytoskeleton. Dephosphorylation of ERM proteins and merlin allows interdomain association to occur.

Furthermore, phosphorylation of ezrin by the Rho kinase ROCK is required for Rho-induced focal adhesion assembly [35^{*}]. A novel link has been found through the *TSC1* tumor suppressor hamartin, which binds to ERM proteins in their open conformation. Hamartin also activates Rho and thereby promotes the formation of focal adhesions [36^{*}].

Ezrin enhances cell motility in response to hepatocyte growth factor (HGF) treatment as measured by the wound-healing ability of epithelial cells [27]. Interestingly, dominant negative forms of ezrin inhibit the HGF-mediated migration of glioma cells by reducing transforming growth factor- β 2 synthesis [37]. Protein kinase C α (PKC α) promotes cell migration by phosphorylating Thr567 of ezrin, and the ezrin mutant T567A impairs PKC α -mediated motility [38^{*}].

ERM proteins control cell growth through different pathways. Transformation of NIH3T3 cells by the Rho exchange factors Net and Dbl is inhibited by the ezrin mutant T567A, suggesting that ezrin activation is required for the transformation induced by these oncogenes [35^{*}]. Signaling by tyrosine kinases is also involved in the control of cell growth by ezrin. Indeed, mutation of Tyr145 to phenylalanine blocks the proliferation of epithelial cells into a collagen type I matrix (A Gautreau, M Arpin, unpublished data). Phosphorylation of Tyr353 is required for epithelial cell survival through activation of the phosphatidylinositol 3-kinase (PI3-k)/Akt pathway [39^{*}]. Recently, ezrin has been shown to bind and activate the focal adhesion kinase, a central regulator of anchorage-dependent growth that also activates the PI3-k/Akt pathway [40]. Ezrin is also involved in survival of T lymphocytes. The binding of ezrin, but not moesin, to CD95 (APO-1/Fas) renders the cells susceptible to CD95-mediated apoptosis, probably by maintaining a polarized distribution of CD95 in the uropods [41^{*}].

These different studies emphasize the interrelation that exists between the membrane-cytoskeleton linker functions of ERM proteins and their ability to transduce signals. We have observed that ezrin T567A is poorly phosphorylated on tyrosine residues compared to wild-type ezrin or to ezrin T567D (A Gautreau, M Arpin, unpublished data), suggesting that activation of ERM proteins is required for these proteins to activate downstream signaling pathways.

A role for merlin in cell adhesion is suggested by the observation that overexpression of wild-type or mutant merlin impairs cell adhesion to the substratum and cell motility [42,43]. Both the amount and the phosphorylation of merlin are regulated by cell adhesion and cell-cell contacts [44]. Non-phosphorylated merlin was shown to mediate contact inhibition of growth through its interaction with CD44, the hyaluronan receptor, by integrating signals from the extracellular matrix [26^{*}].

Overexpression of merlin inhibits Rac-mediated cell transformation and reduces the activity of JNK (c-jun-N-terminal kinase) and Jun/Fos-mediated transcription [22^{**}]. The phosphorylation of Ser516 induced by Rac inactivates merlin by weakening its self-association as well as its interaction with the cytoskeleton.

The hepatocyte growth factor-regulated tyrosine kinase substrate appears to be an effector of merlin. This protein interacts with the open form of merlin and, when overexpressed, inhibits cell growth and motility [45].

Altogether, these experiments indicate that interdomain association plays a role in the regulation of merlin-induced growth. In agreement with this, it has been shown that overexpression of merlin isoform 1 inhibits rat schwannoma cell growth *in vitro* and *in vivo*, whereas merlin isoform 2,

which has no C-ERMAD, does not [46]. The interaction between the C-ERMAD of *Drosophila* NF2, merlin, and the FERM domain of ezrin is also important in the regulation of cell proliferation [47*].

Merlin and ERM proteins in tumor development

The *NF2* tumor suppressor gene displays bi-allelic inactivation in both sporadic and inherited schwannomas. Mouse models have been developed to study the NF2 syndrome experimentally. Homozygous knockout embryos died early during development [48], whereas heterozygous mice were viable but prone to develop a variety of tumors, mostly osteosarcomas [49]. To accurately model the human disease, a conditional knockout has been created using a Schwann cell specific promoter to drive the expression of the Cre recombinase [50*]. These homozygous knockout mice develop schwannomas. Surprisingly, schwannomas have also been obtained in transgenic mice overexpressing, in Schwann cells, a mutant form of merlin that contains an interstitial deletion in the FERM domain. This suggests that this mutant has oncogenic potential [51*], although this mutant is misfolded [5*] and is rapidly degraded by the ubiquitin-proteasome pathway (A Gautreau, J Manent, M Giovannini, M Arpin, unpublished data). Efficient degradation of merlin mutants probably prevents their dominant-negative effects and might explain why the *NF2* gene is a tumor suppressor and not an oncogene.

There is also increasing evidence that ezrin has a role in tumor progression. Comparison of the ezrin level in metastatic cells versus their non-metastatic counterparts revealed a large increase in ezrin expression in metastatic cells from different origins [52–54]. A survey of human tumors also revealed a high expression level of ezrin in invasive cells [55–57]. The rather specific role of ezrin in cell invasion and metastasis that emerges probably involves its different roles in cell motility [27,37,38*], survival [39*,41*] and proliferation [35*] identified in cultured cells. No mutations have been identified so far in the ezrin gene, however, suggesting that this gene is not, in the strictest sense, an oncogene. Only one chromosomal abnormality involving an ERM gene has been observed in a case of anaplastic large cell lymphoma. In this case, a fusion protein between the first 448 amino acids of moesin and 557 amino acids of anaplastic lymphoma kinase containing the kinase domain was detected [58].

Conclusions

ERM proteins and merlin might represent two facets in the control of cell growth mediated by the organization of cortical actin. Merlin acts as a suppressor of cell growth whereas the role of ezrin in promoting cell growth and invasiveness is emerging. Recent studies have highlighted the importance of the conformational activation of these proteins in the transduction of growth signals. Both activating signals and downstream effectors of ERM proteins and merlin are being quickly identified and should provide important insights into the close relationship

that exists between cell morphology and signaling in the process of tumor progression.

Update

Recent papers report that ERM proteins colocalize with CD43 in a membrane domain distal to the immunological synapse [59,60,61]. Binding of ERM proteins to CD43 is required for exclusion of CD43 from the immunological synapse [59,61]. Moreover, dominant-negative ezrin inhibits T cell activation [60,61]. These results suggest that the relocalization of CD43, mediated by ERM proteins, is required for T cell activation.

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