ERM PROTEINS AND MERLIN: INTEGRATORS AT THE CELL CORTEX

Anthony Bretscher[*], *Kevin Edwards*[‡] *and Richard G. Fehon*[§]

A fundamental property of many plasma-membrane proteins is their association with the underlying cytoskeleton to determine cell shape, and to participate in adhesion, motility and other plasma-membrane processes, including endocytosis and exocytosis. The ezrin–radixin–moesin (ERM) proteins are crucial components that provide a regulated linkage between membrane proteins and the cortical cytoskeleton, and also participate in signal-transduction pathways. The closely related tumour suppressor merlin shares many properties with ERM proteins, yet also provides a distinct and essential function.

APICAL DOMAIN
The area of an epithelial cell that faces the lumen.

BASOLATERAL DOMAIN
The area of an epithelial cell that adjoins underlying tissue.

MICROVILLI
Small, finger-like projections (1–2 μm long and 100 nm wide) that occur on the exposed surfaces of epithelial cells to maximize the surface area.

**Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853, USA.*
‡Department of Biological Sciences, Illinois State University, Normal, Illinois 61790, USA.
§Developmental, Cell and Molecular Biology Group, Department of Biology, Duke University, Durham, North Carolina 27708, USA.
Correspondence to A.B.
e-mails: apb5@cornell.edu; kaedwar@ilstu.edu; rfehon@duke.edu
doi:10.1038/nrm882

The ability to polarize is a fundamental property of cells — for example, to break the symmetry of a sphere to select an axis for cell division, to establish functionally distinct APICAL and BASOLATERAL DOMAINS, or to define a front and a back of a motile cell. Polarity is achieved by interpreting signals at the plasma membrane that are derived from either within or outside the cell, which leads to the reorganization of the cytoskeleton and internal membranes. Ultimately, this creates structurally and functionally distinct cortical domains — integrated structures that comprise the plasma membrane and underlying cytoskeleton. A central question in cell biology is how cells assemble and maintain distinct cortical domains, and how these domains are integrated with signalling pathways and membrane transport.

The ezrin–radixin–moesin (ERM) proteins[1] are a family of widely distributed membrane-associated proteins that regulate the structure and function of specific domains of the cell cortex (FIG. 1). Ezrin was originally identified as a component of structures at the cell surface that contain an actin cytoskeleton, such as MICROVILLI and MEMBRANE RUFFLES[2,3], and as a substrate of specific protein tyrosine kinases[4]. It is highly enriched in microvilli on the apical side of polarized epithelial cells[5] (FIG. 2a). Radixin was isolated from liver-cell ADHERENS JUNCTIONS[6], but seems to be primarily concentrated in the microvilli of BILE CANALICULI[7]. Moesin was identified as a protein that binds heparin, a GLYCOSAMINOGLYCAN[6,8]. These three proteins are very closely related (FIG. 3), and are members of the band 4.1 superfamily by virtue of the presence of a shared FERM (Four-point one, ezrin, radixin, moesin) domain at the amino terminus[9–11] (BOX 1).

ERM proteins provide a regulated linkage from filamentous (F)-ACTIN in the cortex to membrane proteins on the surface of cells. Regulated attachment of membrane proteins to F-actin is essential for many fundamental processes, including the determination of cell shape and surface structures, cell adhesion, motility, CYTOKINESIS, PHAGOCYTOSIS and integration of membrane transport with signalling pathways. It is therefore not surprising that ERM proteins have been implicated not only in cell-shape determination but also in membrane-protein localization, membrane transport and signal transduction, which places ERM proteins at a crucial juncture in the integration of cortical functions.

In 1993, the *Neurofibromatosis 2* (*Nf2*) tumour-suppressor gene was identified, and its product, merlin (also known as schwannomin), was found to have a high degree of sequence similarity to the ERM family[12,13]. This similarity immediately raised the possibility that they had overlapping functions with the ERM proteins (reviewed in REFS 14,15). Whereas it is clear that the roles of ERM proteins and merlin do overlap, merlin also has distinct functions, as exemplified by genetic analysis and by its distinct subcellular localization in insect cells (FIG. 2b and c). In this review,

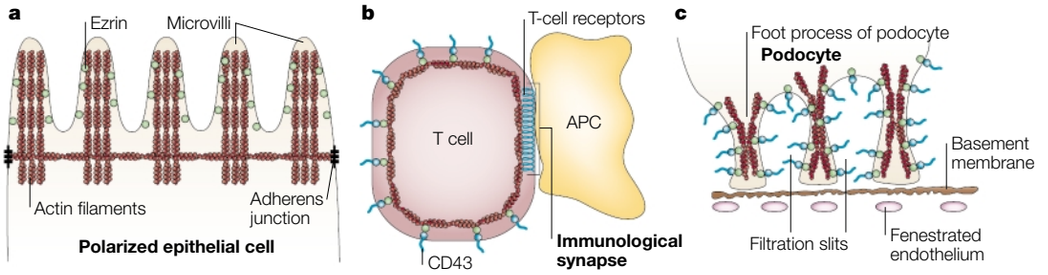


Figure 1 | **ERM proteins contribute to the functional organization of specific plasma-membrane domains.** Examples of three cell types are shown. **a** | The apical aspect of a polarized epithelial cell in which ezrin links actin filaments laterally to the plasma membrane specifically in the microvilli is shown[5]. The identity of membrane proteins with which ezrin associates is not yet clear. **b** | A T cell is shown forming an immunological synapse with an antigen-presenting cell (APC). ERM proteins are believed to be necessary for exclusion of the glycoprotein CD43 from the immunological synapse (reviewed in REF. 72). **c** | The filtration barrier in the kidney glomerulus, in which the filtration slits between podocyte foot processes are believed to be maintained by restriction of podocalyxin to the apical membrane by ezrin[90]. In at least the epithelial-cell and kidney-podocyte systems, the PDZ-containing proteins EBP50 or E3KARP are associated with ezrin and might contribute to the membrane linkage. E3KARP, sodium–hydrogen exchanger type 3 kinase A regulatory protein; EBP50, ERM-binding phosphoprotein 50; ERM, ezrin–radixin–moesin; PDZ, Psd-95, discs-large and ZO-1.

MEMBRANE RUFFLES
Processes that are formed by the movement of lamellipodia that are in the dynamic process of folding back onto the cell body from which they previously extended.

ADHERENS JUNCTION
A cell–cell and cell–extracellular-matrix adhesion complex that is composed of integrins and cadherins that are attached to cytoplasmic actin filaments.

BILE CANALICULUS
A groove on the surface of the liver cell that acts as a collecting system for bile that is made by the cell.

GLYCOSAMINOGLYCANS
Heteropolysaccharides that contain an *N*-acetylated hexosamine in a characteristic repeating disaccharide unit. The repeating structure of each disaccharide involves alternate 1,4- and 1,3-linkages that consist of either *N*-acetylglucosamine or *N*-acetylgalactosamine.

F-ACTIN
(Filamentous actin). A flexible, helical polymer of G-actin (globular actin) monomers that is 5–9 nm in diameter.

CYTOKINESIS
The process of cytoplasmic division.

PHAGOCYTOSIS
An actin-dependent process, by which cells engulf external particulate material by extension and fusion of pseudopods.

PHYLOGENETIC ANALYSIS
The study of evolutionary relationships among organisms.

PHOSPHOROTHIOATE ANTISENSE OLIGONUCLEOTIDES
Short, non-degradable antisense oligonucleotides that bind to specific messenger RNAs and suppress their translation, which inhibits synthesis of specific proteins.

DOMINANT-NEGATIVE
The effect of a defective protein that retains interaction capabilities and so distorts or competes with normal proteins.

we provide an overview of the roles of the ERM and merlin proteins in the biology of the cell cortex (more comprehensive reviews are available elsewhere[15–17]). We focus on both the shared and distinguishing features of merlin and ERM proteins.

Insights from genomics and genetics

Ezrin, radixin and moesin, together with merlin, comprise one class of the FERM superfamily (BOX 1). No other members of the ERM/merlin class are apparent among the mammalian genome sequences that are available at present. The FERM domains of ERM homologues from sea urchin, *Caenorhabditis elegans*, *Drosophila melanogaster* and vertebrates show about 74–82% identity and about 60% identity to those of merlin. As can be seen from the PHYLOGENETIC ANALYSIS (BOX 2), this level of identity is exceptionally high, and indicates that their structure has been remarkably well conserved. The most divergent ERM proteins are found in parasites (tapeworms and schistosomes), which have FERM domains that show only 44–58% similarity to their vertebrate homologues. In addition to the ERM/merlin proteins, more than a dozen other classes of FERM proteins have been described (BOX 1). Despite their diverse structures, many of these proteins are known or suspected to act at the interface of cell signalling and the cytoskeleton. This dual role is best understood for the ERMs, and so they provide an example of how other, less extensively studied, FERM proteins might function.

The ERM proteins apparently arose by gene duplication within the vertebrates, and the high degree of structural conservation among them (FIG. 3) indicates that they might have similar, or indeed redundant, functions. Consistent with this notion, a knockout mutation in the mouse *moesin* gene does not have any discernable phenotype[18]. In addition, PHOSPHOROTHIOATE ANTISENSE OLIGONUCLEOTIDE treatment to reduce ERM protein synthesis in cultured cells indicates that ERM proteins might have at least partially overlapping functions[19]. This redundancy complicates genetic analysis in mammals, and so far has prevented straightforward loss-of-function genetic analysis in the mouse. In principle, however, it should be possible to generate a potentially lethal triple-knockout mouse in which all ERM protein function is eliminated. Preliminary studies in *Drosophila*, which has only one ERM gene — *moesin* — indicate that ERM-protein function is essential for viability and epithelial integrity (O. Nikiforova and R. G. F., unpublished observations). *C. elegans* also has a single ERM gene, although no functional analysis of this gene has been published so far. Interestingly, no FERM-domain-encoding genes have been identified in the genome of the yeast *Saccharomyces cerevisiae*, which implies that FERM domains evolved in response to multicellularity, rather than as a cytoskeletal component (BOX 2).

As a consequence of the absence of readily available genetic tools for the functional analysis of ERM proteins, most studies in mammalian cells have used putative DOMINANT-NEGATIVE and activated constructs. In particular, truncations that comprise only the amino-terminal FERM domain have been used to generate dominant-negative effects[20–22]. It is assumed that expression of any one of these truncated proteins interferes with the functions of all three because of the high degree of conservation of this domain between the ERM proteins. Likewise, expression of an activated form of an ERM protein (for example, moesin T558D, in which T denotes threonine and D denotes aspartic acid; see below) should confer a dominant-active phenotype. In principle, these experiments can be informative with regard to uncovering ERM cellular functions. However, as discussed later, it is not clear that the amino-terminal truncation has a dominant-negative effect on all functions, and for this reason these experiments must be interpreted with caution.

Unlike the ERM proteins, mutational analysis of the product of the *NF2* gene, merlin, has been relatively straightforward. Although merlin is more similar to the

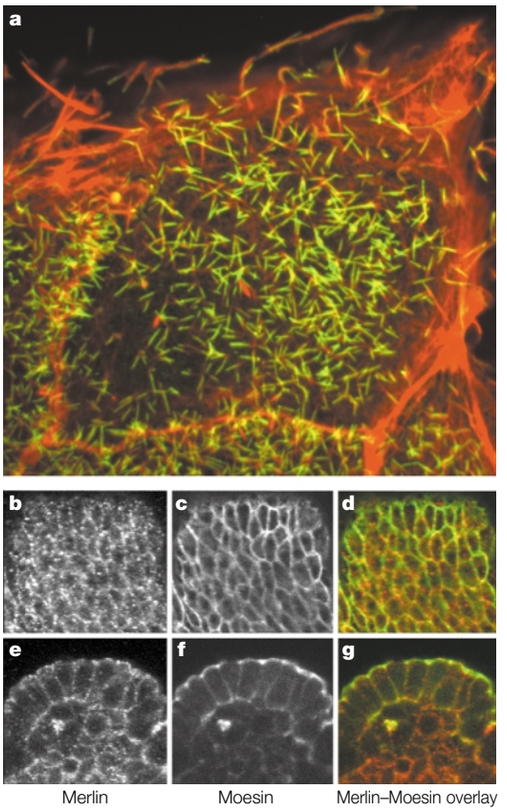


Figure 2 | **Localization of ERM proteins and merlin.** **a** | Localization of ezrin (green) and actin (red) to the microvilli of porcine kidney LLC-PK1 cells. A maximum projection of a stack of focal planes through the whole depth of the cell is shown. **b**–**g** | Co-immunofluorescence staining showing merlin and moesin in *Drosophila melanogaster* embryonic epidermal cells. Panels **b**–**d** show tangential sections through the apical domains of epithelial cells, whereas **e**–**g** show deeper cross-sections of these cells. Merlin (**b** and **e**) is enhanced apically, but is punctate and is found both at the plasma membrane and in the cytoplasm. By contrast, moesin (**c** and **f**) is found primarily associated with the apical plasma membrane. Here, it appears more evenly distributed than merlin. In addition, merlin is more abundantly expressed in the underlying mesodermal cells than is moesin (compare panels **e** and **f**). In **d** and **g**, moesin is shown in green and merlin in red.

ERM proteins than to any other members of the FERM superfamily, it does not seem to have PARALOGUES in humans, mice or *Drosophila*. Mice or flies that are homozygous for mutations in the *NF2* gene are not viable[23,24]. In humans, the Neurofibromatosis type 2 (NF2) disease is dominantly inherited. Individuals who have this disease are believed to inherit one defective copy of *NF2* from one parent and acquire a mutation in the second, wild-type copy at some point later in life. This 'second-hit' mutation is believed to occur in individual SCHWANN CELLS. These cells ultimately give rise to tumours that are characteristic of the disease[25]. Taken together, these results indicate that merlin has essential functions that do not overlap with those of the ERM proteins. Even so, the high degree of structural similarity between these proteins implies that they might be functionally related.

PARALOGUES
Homologous genes that originated by gene duplication (for example, human α-globin and human β-globin).

SCHWANN CELLS
Cells that produce myelin and ensheath axons in the peripheral nervous system.

BLOT OVERLAY
A method used to detect specific protein–protein interactions; protein mixtures are separated by gel electrophoresis, transferred to a membrane and then probed with a labelled test protein. The test protein binds its specific partner on the membrane and can then be detected by its label.

Conformational regulation of ERM proteins

ERM proteins are negatively regulated by an intramolecular interaction between the amino- and carboxy-terminal domains that masks at least some sites of protein interaction. Activation therefore requires separation of the two domains (FIG. 4).

Evidence for an intramolecular association. The initial clue for this model came indirectly from the finding that moesin–ezrin heterodimers can be isolated from cultured cells and that ERM proteins can homo- and heterodimerize, as detected by BLOT OVERLAYS[26]. This provided an assay for identifying the interacting regions, and led to the discovery that the amino-terminal domain can bind with high affinity to the carboxy-terminal ~90 residues of any ERM member; these regions were therefore called N- and C-ERMAD (ERM association domain)[27]. The N-ERMAD was found to be coincident with the FERM domain, and the C-ERMAD can be regarded as another ligand for the FERM domain (TABLE 1). Further studies showed that the bulk of ezrin in the cytoplasm exists as a monomer, as the FERM domain and the C-ERMAD interact with each other[28,29]. In this state, the F-actin binding site is masked, so it was proposed that 'active' molecules would require disruption of the intramolecular association[27]. Results with radixin were also consistent with this model[30]. In striking agreement with the *in vitro* studies, high-level expression of the carboxy-terminal domain of ezrin or radixin induces long membrane-surface extensions, presumably in response to an excess of exposed F-actin binding sites. In some cases, this phenotype can be suppressed by overexpression of the amino-terminal fragment, which supports the mutual-masking model[31,32]. Moreover, expression of only the FERM domain has been proposed to act in a dominant-negative fashion to reduce the presence of cell-surface microvilli[22].

The conformational masking model was firmly established by the demonstration that the binding site for ERM-binding phosphoprotein 50 (EBP50)/NHE-RF (regulatory co-factor of the sodium–hydrogen exchanger isoform 3, (NHE-3)); see below) on the FERM domain is also masked when associated with the C-ERMAD[33]. Although the binding sites for several ligands are thought to be masked in the dormant ERM monomer, this has been shown only for EBP50 and the Rho guanine-nucleotide-dissociation inhibitor (RhoGDI), a negative regulator of Rho[33,34]. Recently, electron micrographs of rotary shadowed images of radixin have shown a compact globular structure for the dormant molecule and an elongated structure for the active molecule, in which two globular domains are connected by a thin filamentous region[35].

In addition to monomers, cells contain dimers (homo- and hetero-) and higher-order oligomers of ERM proteins. At present, the relevance of the oligomeric species is unknown, especially as increased formation of ezrin-containing cell-surface structures in different settings has been reported to correlate with both an increase[28] and a decrease[36] in the dimer:monomer ratio.

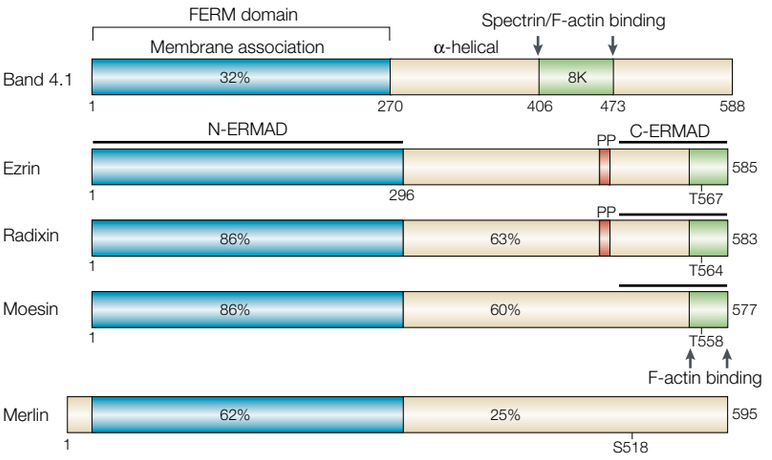


Figure 3 | **Domain relationships between erythrocyte band 4.1, the ERM family and merlin.** The amino-terminal regions contain a ~300 residue FERM domain. Sequence identity to ezrin is shown. After the FERM domain, Band 4.1 shows no significant sequence identity with the ERM proteins or merlin; ERM proteins show very high sequence identity, whereas merlin is more divergent. ERM proteins and merlin can all form an intramolecular association through their N-ERMAD/FERM domains and C-ERMADs, and, where tested, between ERM members and merlin. ERM members have a carboxy-terminal filamentous (F)-actin binding site, whereas merlin does not. The region between the FERM domain and C-ERMAD is predicted to have a high propensity to form an α-helical coiled-coil, although the functional significance of this has not been determined. Mammalian ezrin and radixin have a region that is rich in prolines (PP), the function of which is unknown, whereas moesin does not. The position of the carboxy-terminal threonine (T; ERM proteins) or serine (S; merlin), the phosphorylation of which reduces the FERM–C-ERMAD intramolecular interaction, is indicated. C-ERMAD, carboxy-ERM association domain; ERM, ezrin–radixin–moesin; FERM, Four-point one, ezrin, radixin, moesin; N-ERMAD, amino-ERM association domain.

Regulation by phosphorylation. So, how does the C-ERMAD dissociate from the FERM domain? Enhanced phosphorylation of threonine 558 (T558) of moesin was originally noted during PLATELET activation[37], and phosphorylation of the corresponding residue in all ERM proteins was subsequently found to reduce the affinity of the C-ERMAD for the FERM domain[38]. Moreover, phosphorylated ERM proteins are found selectively in cell-surface structures[39,40], and transfection of cells with T567D ezrin — a mutant construct that mimics the phosphorylated molecule — can induce the formation of abundant cell-surface structures, presumably by creating many more F-actin-membrane linkages[36]. At least three kinases have been shown to phosphorylate this conserved threonine residue *in vivo* or *in vitro* — Rho kinase[38,41] (see below), protein kinase Cα (PKCα)[42] and PKCθ[43,44]. Phosphorylation of moesin or ezrin by PKCθ in the presence of lipids unmasks the F-actin and EBP50-binding sites[44], which adds support to the conformational activation model. However, recent work has shown that pathways other than carboxy-terminal threonine phosphorylation can lead to the activation of ERM proteins[45].

The role of lipids. As indicated above, lipids have also been implicated in ERM activation. Initial evidence for this came from the finding that a truncated protein that contains only the amino terminus of ezrin, radixin or moesin interacts with the cytoplasmic tail of the hyaluronan receptor CD44 under physiological conditions, whereas full-length ERM proteins require phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) for this interaction[46]. However, it was subsequently found that binding of full-length ERM proteins to the positively charged juxtamembrane region of CD44 did not require PtdIns(4,5)P_2 (REF. 47). Studies in which T558-phosphorylated moesin was isolated from activated platelets showed that PtdIns(4,5)P_2, or some other lipid or detergent, is required to unmask the F-actin binding site *in vitro*[48]. PtdIns(4,5)P_2 has been shown to bind the FERM domain[49] and mutagenesis indicated that lysine residues K63, K64, K253, K254, K262 and K263 in this domain are important for PtdIns(4,5)P_2 binding *in vitro* and ezrin function *in vivo*[50]. Further supporting the *in vivo* involvement of PtdIns(4,5)P_2, microinjection of NEOMYCIN — which titrates out polyphosphoinositides — results in loss of cell-surface microvilli, presumably because PtdIns(4,5)P_2 is needed for ERM protein activation[45].

Structural changes between the dormant and active conformations. Recent X-ray structures have provided considerable insight into the conformation of the ERM proteins in dormant and active states. So far, three structures have been reported: a complex between the FERM domain of moesin (residues 1–297) and its C-ERMAD (residues 467–577)[51]; the FERM domain of radixin (residues 1–310) with and without complexed inositol trisphosphate ($InsP_3$; REF. 52); and the moesin FERM domain with part of the α-helical central domain (residues 1–346)[53]. A comparison of these structures provides the first insight into a FERM domain and how the C-ERMAD binds to and modifies its structure.

The globular FERM domain is composed of three subdomains (F1, F2 and F3) that are arranged like a clover leaf. Although no sequence conservation is evident, all three subdomains have structural homology to previously described folds (BOX 1). The F1 domain of moesin (residues 4–82) is very similar to ubiquitin, F2 (residues 96–195) has structural similarity to acyl-CoA binding protein and F3 (residues 204–297) shows structural homology to an adaptable module that is variously described as a phosphotyrosine-binding (PTB), a PLECKSTRIN-HOMOLOGY (PH) or an Enabled/VASP-1 (EVH1) domain. This versatile domain has been found to bind peptide and lipid ligands in both signalling and cytoskeletal proteins.

In contrast to the globular FERM domain, the carboxy-terminal ~80-residue domain adopts an extended structure and is composed of one β-strand and six helical regions that bind to and cover an extensive area on the FERM-domain surface[51]. This extended conformation is almost certainly dictated by the FERM-domain surface, and there are probably very different conformations for this region depending on whether it is free or bound to F-actin. Interestingly, positively charged amino acids in the extreme carboxy-terminal region that are believed to be involved in F-actin

PLATELET
The smallest blood cell, which is important in haemostasis and blood coagulation.

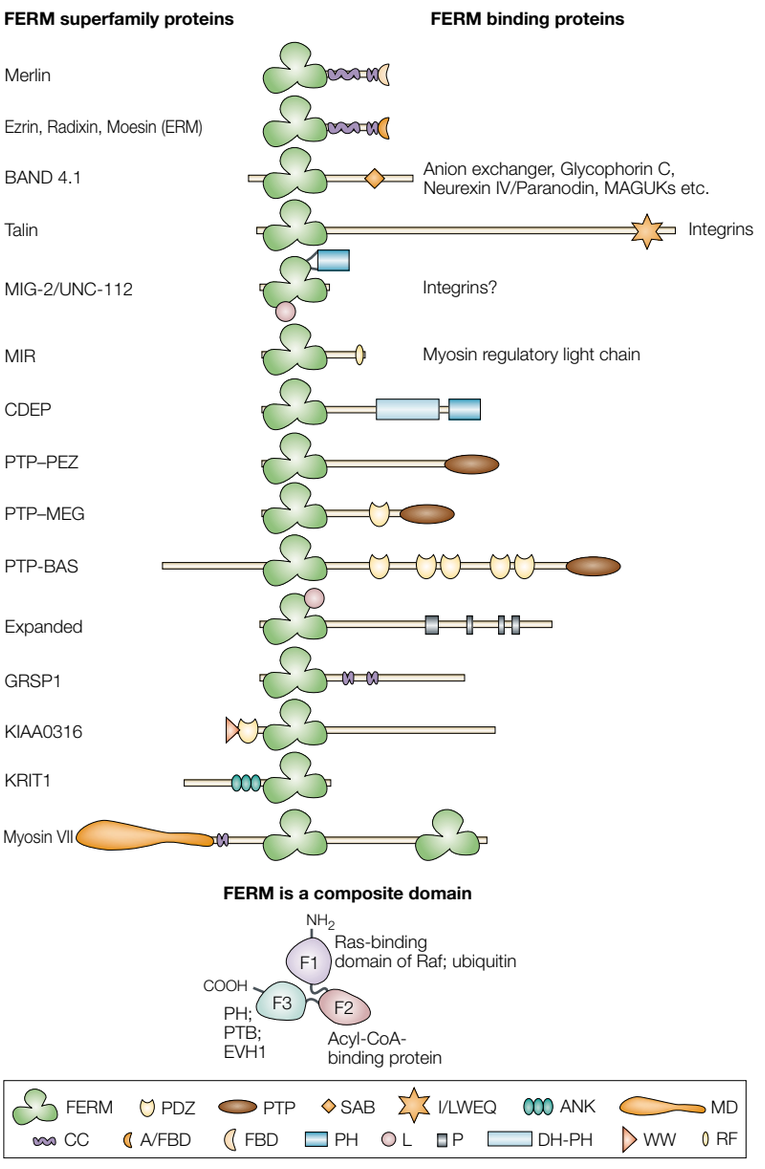
NEOMYCIN
An antibiotic complex that binds polyphosphoinositides.

PLECKSTRIN HOMOLOGY (PH) DOMAIN
A sequence of 100 amino acids that is present in many signalling molecules and binds to lipid products of phosphatidyl-inositol 3-kinase. Pleckstrin is a protein of unknown function that was originally identified in platelets. It is a principal substrate of protein kinase C.

Box 1 | **FERM domains**

FERM (Four-point one, ezrin, radixin, moesin) domains[120] occur in numerous membrane-associated signalling and cytoskeletal proteins, in which they act as multifunctional protein- and lipid-binding sites. Generally located at or near the amino terminus of a protein, they are connected by linkers to various domains.

The upper part of the figure shows a domain map of FERM superfamily proteins (A/FBD, ERM actin and FERM-binding domain; ANK, ankyrin repeat region; CC, coiled-coil; DH–PH, Dbl homology–pleckstrin homology (RhoGEF) domain; FBD, FERM-binding domain; I/LWEQ, talin actin binding domain (in which E denotes glutamic acid; I, isoleucine; L, leucine; Q, glutamine; W, tryptophan); L, extended loop situated in the indicated FERM subdomain; MD, myosin motor domain; P, proline-rich region; PDZ, PSD95/Dlg/ZO1-homologous peptide binding domain; PH, pleckstrin-homology domain; PTP, protein tyrosine phosphatase domain; RF, RING finger; SAB, 4.1 spectrin-actin binding domain; WW, WW domain.) Some known FERM-domain binding proteins are indicated on the left.

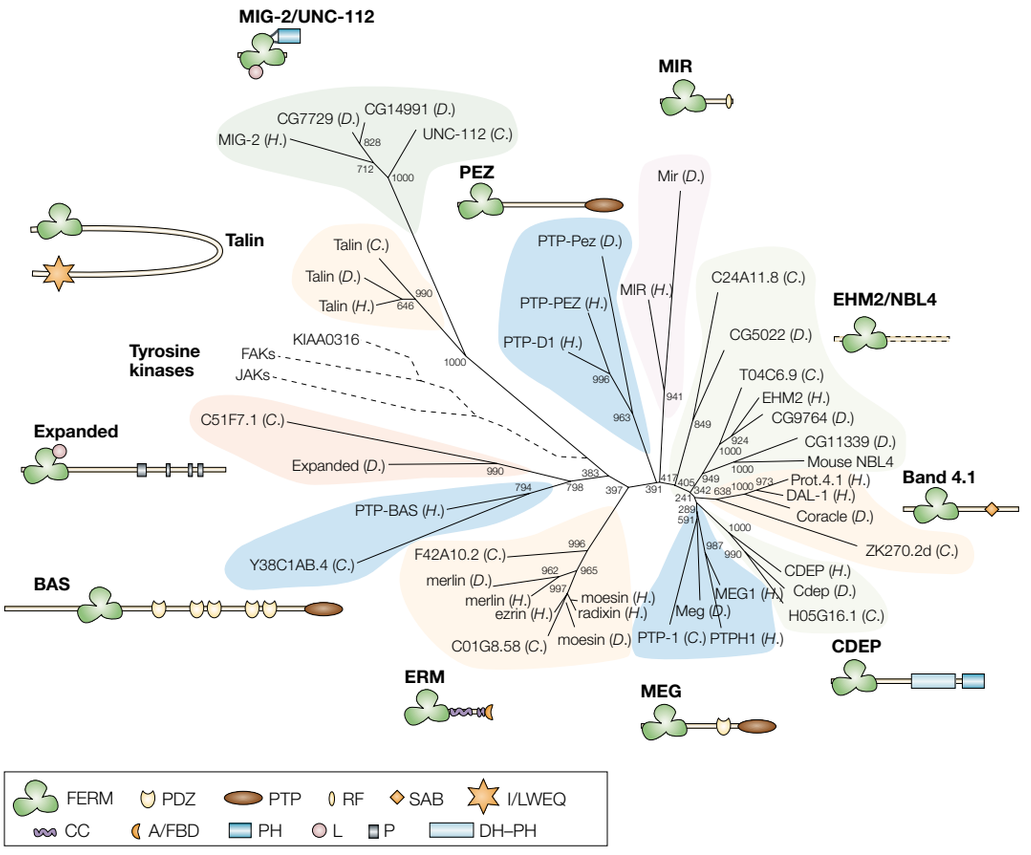


The lower figure is a schematic of the moesin crystal structure. F1, F2 and F3 indicate FERM subdomains; other proteins/domains that share each subdomain's fold are listed[51]. EVH, Enabled/VASP-1 domain; MAGUK, membrane-associated guanylate kinase; PTB, phosphotyrosine-binding domain.

binding are buried in the FERM–C-ERMAD interface, which explains why the F-actin binding site is masked in the dormant monomer.

The structures of the radixin FERM domain with and without bound $InsP_3$ are almost identical[52]. So, the free FERM domain probably represents this domain in an active conformation. $InsP_3$ binds in a basic groove between subdomains F1 and F3, K60 and K63 in F1, and K278 in F3 make contacts with the phosphates. This site is distinct from the residues (K63, K64, K253, K254, K262 and K263) that were previously identified as being important for binding PtdIns(4,5)P_2 *in vitro*[50], so the physiological site of PtdIns(4,5)P_2 interaction remains uncertain. The availability of the structure of the FERM–C-ERMAD complex — which represents the dormant structure of the FERM domain — and that of the free, unmasked, FERM domain allows a comparison of structural changes that are induced when the C-ERMAD binds. These comparisons indicate that release of the C-ERMAD from the FERM domain results in a significant conformational change in the F3 domain. Specifically, the terminal β-strand of four β-sheets, which connects to a long α-helix, moves about 6 Å. The radixin FERM domain with $InsP_3$ bound in a cleft between the F2 and F3 domains adopts a similar conformation, which indicates that

Box 2 | **FERM domain phylogeny**



Phylogenetic analysis of representative human, *Drosophila* and worm FERM (Four-point one, ezrin, radixin, moesin) domains shows which FERM proteins are orthologues, and the relationship between orthologous groups (*C.*, *C. elegans*; *D.*, *Drosophila*; *H.*, human). The tree was constructed using web-based versions of CLUSTALW and DRAWTREE[121] (see online links), from a manual alignment of 42 superfamily members in the region that corresponds to the crystallized portion of human moesin (residues 1–297). Bootstrap values, obtained from the CLUSTALTREE program and indicated at most nodes, denote the number of times a grouping occurs out of 1,000 random samples from the alignment. The JAK/FAK/KIAA0316 portion of the superfamily was omitted from the alignment due to its extremely low sequence similarity to the proteins shown, but its placement is indicated (dotted lines) on the basis of a similar tree by Girault *et al.*[122]. The sequences cluster into 14 distinct orthologous groups, and most groups have representatives from all three phyla. So, the original FERM protein must have undergone a striking adaptive expansion in the earliest metazoans. Proteins that have similar tails are always (as opposed to sometimes, as might be expected) clustered together, even though the tail sequences were not used to build the phylogeny. This implies that the FERM-tail associations in each class are ancient and/or highly specialized.

The orthologous groups partition into 11 classes, three of which contain actin-binding proteins (Band 4.1, ERM/merlin, talin; orange regions). Three are FERM domains that are associated with protein tyrosine phosphatases ('FERM-PTPs': BAS, phosphatase derived from basophilic leukaemia cell line); PEZ (phosphatase with ezrin-like domain), MEG (phosphatase derived from megakaryoblastic cell line)[123]; blue regions), and the remaining classes are less well characterized. Interestingly, functionally related classes (actin-binding proteins, phosphatases) do not cluster together. This is surprising, as a single origin for the FERM-PTPs is proposed by the observation that their PTP domains cluster together within the PTP superfamily[124]. One possibility is that the FERM domains of each class have diverged sharply as they adapted to bind new, unrelated ligands and this obscures their early evolutionary relationships. Alternatively, the membrane–cytoskeletal linker functions and FERM-PTPs might have arisen independently two or three times during metazoan evolution. A/FBD, ERM actin and FERM binding domain; CC, coiled-coil; DH–PH, Dbl homology–pleckstrin homology (RhoGEF) domain; I/LWEQ, talin actin binding domain (in which E denotes glutamic acid; I, isoleucine; L, leucine; Q, glutamine; W, tryptophan); L, extended loop situated in the indicated FERM subdomain; MD, myosin motor domain; P, proline-rich region; PDZ, PSD95/Dlg/ZO1-homologous peptide binding domain; PH, pleckstrin-homology domain; PTP, protein tyrosine phosphatase domain; RF, RING finger; SAB, 4.1 spectrin actin binding domain.

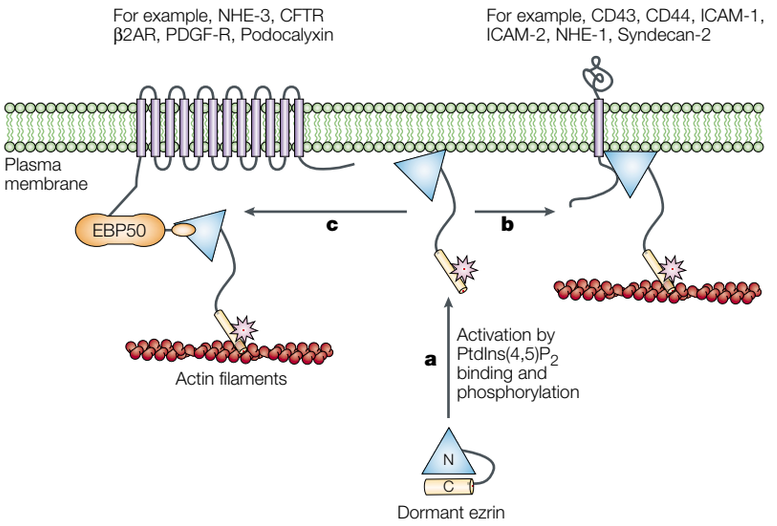


Figure 4 | **A model for the activation and function of the ERM proteins.** ERM proteins exist in a dormant, monomeric form in which the FERM/N-ERMAD domain is associated with the C-ERMAD (**a**). Local production of phosphatidylinositol 3,4-bisphosphate (PtdIns(4,5)P_2) recruits ERM proteins to the plasma membrane, which places them in a location to be phosphorylated, and thereby activated, by Rho-kinase or protein kinase Cθ. Activated ERM proteins can then participate in microfilament–membrane linkage by direct association with transmembrane proteins (**b**), or indirectly through scaffolding molecules such as EBP50/NHE-RF and E3KARP (**c**). ERM proteins also participate in signal-transduction pathways (not shown). B2AR, β_2-adrenergic receptor; C-ERMAD, carboxy-ERM-association domain; CFTR, cystic fibrosis transmembrane conductance regulator; E3KARP, NHE type 3 kinase A regulatory protein; EBP50, ERM-binding phosphoprotein 50; ERM, ezrin–radixin–moesin; FERM, Four-point one, ezrin, radixin, moesin; ICAM, intercellular adhesion molecule; NHE, sodium–hydrogen exchanger; NHE-RF, regulatory co-factor of NHE-3; PDGF-R, platelet-derived growth factor receptor; PKC, protein kinase C. Adapted with permission from REF. 15. ©2000 Annual Reviews.

binding of PtdIns(4,5)P_2 might induce conformational strain in the F3 domain that is transmitted, through the long α-helix that it contacts, to the β-sheet. This region of the F3 subdomain makes important contacts with the last helix of the C-ERMAD. So, it is possible that $InsP_3$ binding between F2 and F3 might induce a conformational change to reduce the affinity of the C-ERMAD for the FERM domain. However, there is one caveat — the FERM domain can bind PtdIns(4,5)P_2 when the C-ERMAD is bound[49], which again raises the question of whether $InsP_3$ lies in a physiologically relevant PtdIns(4,5)P_2 binding site.

The regulatory threonine in the C-ERMAD — T558 in moesin — is predicted to induce a significant conformational change in the FERM–C-ERMAD interface when it is phosphorylated. T558 is buried in the interface, so that the addition of a phosphate group cannot be accommodated without a conformational change. In addition, juxtaposition of the negatively charged phosphate group and the negatively charged FERM-domain surface would be unfavourable, which lends support to the model that phosphorylation would weaken the C-ERMAD–FERM association[51].

The concept that intramolecular interactions provide a conformational mechanism for regulating the biological activity of a protein is proving to be quite a general phenomenon for both signalling and cytoskeletal proteins. Janus kinase 3 (JAK3) and protein-tyrosine phosphatase derived from megakaryoblastic cell line (PTP-MEG) provide other examples of this in the band 4.1 superfamily. The FERM and tyrosine-kinase domains of JAK3 can associate, and structural mutations in the FERM domain inhibit tyrosine-kinase activity, which results in severe combined immunodeficiency in human patients[54]. For PTP-MEG (BOX 1), proteolytic release of the FERM domain activates the protein tyrosine phosphatase activity[55]. Examples outside the band 4.1 superfamily include vinculin[56,57], the Wiskott–Aldrich syndrome protein (WASP)/Scar family[58] and formins[59].

Microfilament–membrane linking

On the basis of its enrichment and localization in cell-surface structures, ezrin was originally proposed to be a microfilament–membrane linking protein, yet no significant F-actin binding activity could be detected in the isolated protein[2,60]. ERM proteins were later shown to contain an F-actin binding site within their carboxy-terminal 30 residues[38,61–63], but this is masked in the isolated dormant monomer[27]. More actin-binding sites have been described[64], but their physiological relevance remains to be clarified[16,65].

The amino-terminal FERM domain was proposed to interact with membrane proteins on the basis of the membrane-binding properties of the founding FERM family member band 4.1 (REF. 66), and this has indeed been shown to be the case. Two types of interaction with membrane proteins have been documented (FIG. 4 and TABLE 1): a direct association of the FERM domain with the cytoplasmic tails of transmembrane proteins, and an indirect association with the tail of membrane proteins through two related SCAFFOLDING PROTEINS — EBP50/NHE-RF and NHE type 3 kinase A regulatory protein (E3KARP) — that contain PDZ DOMAINS.

Direct association with membrane proteins. Many of the direct interactions between ERM proteins and transmembrane proteins involve adhesion receptors. ERM proteins were first found to interact with the receptor for hyaluronate, CD44 (REF. 67), through a positively charged juxtamembrane region in the CD44 tail[47,68]. The ezrin–CD44 interaction is important for directional cell motility, and turnover of phosphorylation at S219 in the tail of CD44 — which reduces the ezrin–CD44 interaction — is crucial for this motility[69]. Ezrin also interacts with the cytoplasmic tail of intercellular adhesion molecule-2 (ICAM-2), an adhesion molecule that has immunoglobulin-like domains[47,70] and this, too, has been shown to be of functional importance. NATURAL KILLER CELLS need to recruit ICAM-2 into a bud-like projection known as a uropod before they can be activated by interleukin-2 binding. This recruitment of ICAM-2 is dependent on ezrin, as cells that lack ezrin have a uniform ICAM-2 distribution[71].

Recently, the significance of other direct linkages has become apparent. For example, T-cell activation requires the removal of the mucin CD43 from the

SCAFFOLDING PROTEIN
A protein that has specific binding sites and is therefore important in the assembly, structure and function of larger molecular complexes.

PDZ DOMAIN
Protein interaction domain that often occurs in scaffolding proteins and is named after the founding members of this protein family (Psd-95, discs-large and ZO-1).

NATURAL KILLER CELLS
A class of lymphocytes that are crucial in the innate immune response. They exert a cytotoxic activity on target cells (for example, virus-infected cells) that is enhanced by cytokines such as interferons.

IMMUNOLOGICAL SYNAPSE
A tight junction between T lymphocytes and target cells.

IMMUNOLOGICAL SYNAPSE between a T cell and an ANTIGEN-PRESENTING CELL (FIG. 1b). This process depends on an interaction between ERM proteins and the cytoplasmic tail of CD43 (REFS 20,72–74), which was already known to bind ERM proteins[47]. T cells that express a dominant-negative form of ezrin (the FERM domain fused to enhanced GREEN FLUORESCENT PROTEIN) fail to exclude CD43 from the synapse and are not activated, which provides a strong case for the functional importance of the interaction[20,74]. Another example is the direct linkage between ezrin and the sodium–hydrogen exchanger isoform 1 (NHE-1)[75]. Introduction of NHE-1 into PS120 fibroblasts that do not usually express this multiple-spanning membrane protein results in cytoskeletal reorganization and the formation of FOCAL CONTACTS. A similar morphological change occurs when an NHE-1 mutant that is defective in proton translocation is introduced into these cells. This shows that NHE-1 has two separable functions — proton translocation and cytoskeletal regulation. The effect on cytoskeletal organization requires a basic region at the extreme terminus of the cytoplasmic tail of NHE-1 that binds ERMs. So, a functional linkage between ERM proteins and NHE-1 — which is independent of the ion-transporting function of NHE-1 — regulates cytoskeletal organization.

Scaffolding-protein-mediated membrane-protein association. In addition to associating directly with the cytoplasmic tails of membrane proteins, the FERM domain interacts strongly with EBP50/NHE-RF and E3KARP. Both these proteins have two PDZ domains and have a carboxy-terminal sequence of 30 amino acids that binds ezrin[33,76]. PDZ domains typically recognize a specific consensus sequence in the extreme carboxyl terminus of their substrates, and the PDZ domains of EBP50/NHE-RF and E3KARP have been proposed to bind a plethora of membrane and cytoplasmic proteins (for review, see REFS 15,77–79). Several functions for EBP50/NHE-RF and E3KARP are emerging. First, they mediate the direct regulation of the activity of ion transporters, notably NHE-3 by protein kinase A (REFS 78,80). In the epithelial cells of the kidney proximal tubule, EBP50/NHE-RF provides a linkage between NHE-3 and ezrin, whereas ezrin, in turn, binds the protein kinase A regulatory subunit, and thereby localizes the kinase[81,82]. Second, EBP50/NHE-RF and E3KARP might have a role in regulating the endocytosis of many plasma-membrane proteins to which they bind (including cystic fibrosis transmembrane conductance regulator[83,84], β2-adrenergic receptor[85–87], NHE-3 (REFS 76,80,88) and platelet-derived growth factor receptor[89]). Third, membrane proteins that are bound through EBP50/NHE-RF or E3KARP and ERM proteins to F-actin might be restricted to specific plasma-membrane domains. In the foot processes of PODOCYTES in the kidney glomerulus (FIG. 1c), the highly charged glycoprotein podocalyxin is localized to the apical membrane that lines the filtration slits in which charge repulsion is believed to contribute to their maintenance. Recently, it has been shown that podocalyxin binds, through its carboxy-terminal DTHL sequence (in which D denotes aspartic acid; T, threonine; H, histidine; L, leucine), to E3KARP, and through ezrin to F-actin[90].

ANTIGEN-PRESENTING CELL
A cell, most often a macrophage or dendritic cell, that presents an antigen to activate a T cell.

GREEN-FLUORESCENT PROTEIN
An autofluorescent protein that was originally identified in the jellyfish *Aequorea victoria*.

FOCAL CONTACT
A small cellular structure that is associated with lamellipodia and pseudopods, in which the extracellular matrix on the outside of the cell is linked to the actin cytoskeleton on the inside of the cell.

PODOCYTE
A fenestrated cell that forms the visceral layer of the Bowman's capsule in the kidneys.

GTPγS
A non-hydrolysable analogue of GTP.

Integration into signalling pathways

ERM proteins also associate with cytoplasmic signalling molecules. ERM proteins seem to lie both downstream and upstream in signal-transduction pathways in epithelia and other tissues.

ERM proteins participate in signalling by Rho and PKC. As described earlier, ERM protein conformation — and therefore activity — is regulated by a combination of phospholipid binding and phosphorylation. Both events have been proposed to lie downstream of signals mediated by Rho[38,91–94]. ERM protein activation seems to have a crucial role in the cellular cytoskeletal response to Rho-pathway activation. Hall and co-workers[95] have shown that Rho-induced cytoskeletal rearrangements are dependent on cytoplasmic pools of ERM proteins. Their work showed that a soluble pool of cytoplasmic ERM proteins redistributes to the plasma membrane in response to Rho activation by GTPγS. In a similar fashion, ERM proteins seem to act as effectors in PKCα and PKCθ signalling events. As described previously, both kinases have been shown to phosphorylate the conserved carboxy-terminal threonine and thereby facilitate conformational activation of ERM proteins[42–44]. In addition, PKC-mediated phosphorylation has been proposed to regulate interactions between CD44 and ezrin by altering phosphorylation of the intracellular domain of CD44 (REF. 69). So, PKC might regulate the effects of CD44 on cell proliferation and migration by controlling phosphorylation of both CD44 itself and the ERM proteins with which it associates.

ERM proteins regulate Rho activity. Intriguingly, recent evidence also places ERM proteins upstream of Rho-pathway activation through direct association with proteins that regulate Rho function. The possibility that ERM proteins function both upstream and downstream of Rho implies there could be a feedback loop for Rho-pathway autoregulation. Most evidence to date indicates that ERM proteins positively regulate Rho activity. RhoGDI, a potent sequestering factor and therefore negative regulator of Rho activation, has been shown to bind to the FERM domain of ERM proteins[34]. This binding is inhibited in dormant ERM proteins. *In vitro* studies indicate that ERM binding to RhoGDI releases inactive Rho from the GDI, and thereby allows activation through the exchange of GDP for GTP. The functional significance of this interaction was tested in Swiss 3T3 cells by expressing only the FERM domain of radixin and using the formation of STRESS FIBRES as an assay for Rho-pathway activation[34]. The results indicated that binding of the FERM domain to RhoGDI upregulates Rho-pathway

Table 1 | **Proteins that bind the FERM domains of ERM proteins and merlin**

Protein	Ezrin/radixin/moesin	Merlin	Reference
Carboxy-terminal association domain (C-ERMAD)			
ERMs	Yes	Yes	27,51,108,110
Merlin	Yes	Yes	27,51,108,110
Adhesion molecules			
CD44	Yes	Yes	47,67,68,129
ICAM-1	Yes	ND	70
ICAM-2	Yes	ND	47,70
ICAM-3	Yes	ND	130
L-Selectin	Yes	ND	131
Ion transporter			
NHE-1	Yes	ND	75
Scaffolding molecules			
EBP50/NHE-RF	Yes	Yes	33,132
E3KARP	Yes	No	33,76,110,132
SAP97	Yes	ND	133
Syndecan-2	Yes	ND	134
Signalling molecules			
RhoGDI	Yes	Yes	34,46,112
Dbl	Yes		135
FAK	Yes	ND	136
PI3K	Yes	ND	137
Hamartin	Yes	Weak	96
HRS	No	Yes	111
Others			
CD43 (leukosialin)	Yes	ND	47,73
CD95 (APO-1/Fas)	Yes	ND	138
βII-spectrin	ND	Yes	113
Palladin	Yes	ND	139

Dbl, diffuse B-cell lymphoma; E3KARP, NHE type 3 kinase A regulatory protein; EBP50/NHE-RF, ERM-binding phosphoprotein 50/regulatory co-factor of the sodium–hydrogen exchanger isoform 3; ERM, ezrin–radixin–moesin; FAK, focal adhesion kinase; FERM, Four-point one, ezrin, radixin, moesin; HRS, hepatocyte growth factor-regulated substrate; ICAM, intercellular adhesion molecule; ND, not determined; NHE-1, sodium–hydrogen exchanger isoform 1; PI3K, phosphatidylinositol 3-kinase; RhoGDI, Rho guanine nucleotide dissociation inhibitor; SAP97, synapse-associated protein 97.

function and, therefore, that ERM proteins positively regulate Rho activity.

However, subsequent studies have proposed that expression of the FERM domain has dominant-negative, rather than activating, effects on ERM protein function (see below)[20–22], and preliminary studies in *Drosophila* using genetic criteria support this interpretation (O. Nikiforova, S. Jong, and R. G. F., unpublished observations). Although it is possible that expression of the FERM domain in cells has activating effects on some ERM functions and dominant-negative effects on others, these considerations indicate that caution should be exercised in interpreting these results. In addition, it is clear that the relationship between ERM proteins and Rho-pathway activation needs further exploration. Genetic studies in *Drosophila* and other model systems, using straightforward loss-of-function genetic analysis, will be important in resolving these questions.

A second line of evidence that indicates that ERM proteins function upstream of Rho comes from YEAST TWO-HYBRID assays. Using the ezrin FERM domain as a probe, a small region of hamartin — the product of the *Tuberous sclerosis 1* (*TSC1*) tumour-suppressor gene — was identified as an ERM-binding protein[96]. Overexpression of full-length hamartin induces activation of the Rho pathway in Swiss 3T3 cells, whereas overexpression of only the ERM-binding domain of hamartin interferes with Rho activation that is induced by LYSOPHOSPHATIDIC ACID (LPA). These results indicate that ERM proteins might positively regulate Rho activity by recruiting hamartin to the cell cortex in response to activation by serum or LPA.

The structure and function of merlin

One of the most striking discoveries in the past decade with regard to ERM proteins and related family members is that the *NF2* tumour-suppressor gene encodes the moesin, ezrin, radixin-like protein 'merlin'/schwannomin[12,13]. The finding that an ERM-related protein could have tumour-suppressor functions was unexpected because neither other family members nor other proteins that are associated with the cytoskeleton had at that time been shown to regulate cell proliferation. This indicated that merlin could differ mechanistically from other known tumour-suppressor genes that are more directly involved in the regulation of cellular proliferation. More importantly, the high degree of structural similarity between merlin and the ERM proteins indicates that these proteins might share regulatory mechanisms and possibly also cellular functions.

So, biochemical studies of merlin structure and function have been based on earlier studies of ERM proteins, whereas genetic analysis of merlin-mutant phenotypes has provided insights into merlin and ERM protein functions.

STRESS FIBRES
Axial bundles of F-actin that underlie the cell bodies.

YEAST TWO-HYBRID
A technique used to test if two proteins physically interact with each other. One protein is fused to the GAL4 activation domain and the other to the GAL4 DNA-binding domain, and both fusion proteins are introduced into yeast. Expression of a GAL4-regulated reporter gene indicates that the two proteins physically interact.

LYSOPHOSPHATIDIC ACID
(LPA). Any phosphatidic acid that is deacylated at positions 1 or 2. It binds to a G-protein-coupled receptor, which results in the activation of the small GTP-binding protein Rho and the induction of stress fibres.

Structural comparisons between ERM proteins and merlin. There are striking structural similarities between merlin and the ERM proteins. Like the ERM proteins, merlin can be divided into three apparent functional domains: the amino-terminal FERM domain, an extended COILED-COIL REGION, and a short carboxy-terminal domain (FIG. 3). Furthermore, the merlin FERM domain forms a three-dimensional structure that is similar to that of ERM proteins[97,98], and interacts with at least some of the same proteins — in particular CD44. Given these findings, it seems probable that merlin function and regulation are fundamentally similar to those of the ERM proteins.

Despite these similarities, there are clear structural differences between merlin and ERM proteins. These differences are most apparent when merlin and ERM sequences from mammals and *Drosophila* are compared[99]. One obvious difference is that merlin lacks the carboxy-terminal F-actin-binding domain that is found in ERM proteins (although this domain does seem to bind the FERM domain like ERM proteins do; see below). Several studies have shown that merlin can instead interact with F-actin through actin-binding sites within the FERM domain[100–102]. The *in vivo* significance of this actin-binding activity has not been examined. However, given the observed differences in subcellular localization between merlin and ERM proteins (FIG. 2; REFS 99,103), it seems unlikely that the amino-terminal actin-binding domain of merlin functions similarly to the carboxy-terminal actin-binding domain of ERM proteins.

Although the FERM domains are quite similar (~ 43% identity across the FERM domains of *Drosophila* and human ERMs and merlin), there are regions in the merlin FERM domain that are conserved between humans and *Drosophila* that are distinct from ERM proteins. Crystallographic analysis of the merlin FERM domain has shown that most divergent residues are found clustered on the surface, which indicates that they might serve as sites for effector binding or regulatory interactions[97,98]. In addition, a seven-amino-acid stretch known as the 'blue box'[104], which is perfectly conserved in *Drosophila* and human merlin — but not in the ERM proteins — probably forms an essential part of the interface between the FERM domain and the tail domain in the folded conformation[97,98]. This region has been shown to have essential functions in both *Drosophila*[104] and humans[105]. These structural differences, within the overall context of remarkable similarity to the ERM proteins, presumably allow merlin-specific protein–protein interactions and regulatory mechanisms. Most of the known disease-causing missense mutations in the FERM domain would seem to disrupt folding of one of the subdomains or interactions between domains, which indicates that the overall three-dimensional structure of the FERM domain is probably essential to merlin function.

Considerable attention has been paid to the notion that merlin, like ERM proteins, might exist in folded and unfolded conformations. In addition to the overall structural similarities between merlin and the ERM proteins, several studies have clearly shown interactions between the merlin amino- and carboxy-terminal domains that are analogous to those that have been described for ERM proteins[106–110]. Consistent with these results, merlin seems to exist in both a folded and an unfolded conformation in solution[100]. Similar to the ERM proteins, only the open conformation interacts with particular ligands, such as the hepatocyte-growth-factor-regulated substrate (HRS)[111]. Interestingly, in mammals (but not *Drosophila*), alternative splicing of the *NF2* gene product gives rise to a second merlin isoform with a unique carboxyl terminus, known as isoform 2. Results from several studies indicate that this isoform exists in a constitutively open conformation[106,108,112,113], and might therefore differ functionally from isoform 1. However, recent work has shown that knockout mice that express only isoform 2 develop normally and mature into healthy adults (M. Giovannini, personal communication).

Regulation of merlin activity. Although there is considerable evidence that the open conformation of ERM proteins represents the 'active' state of the molecule, the evidence that this is also the case for merlin is less clear. Studies in *Drosophila* indicate that merlin mutations that remove the carboxy-terminal tail — and therefore eliminate the possibility of head-to-tail folding — result in a constitutively active form of the protein that provides full genetic rescue of a null merlin mutation[104]. This result clearly indicates that, like ERM proteins, the unfolded form of merlin is active *in vivo*. However, other experiments indicate the opposite. Studies in mammalian cells have shown that the S518 residue (which is near the carboxyl terminus, but is not homologous to T558 in moesin) is phosphorylated by the p21-activated kinase PAK[114,115], and that the S518D mutation — which mimics the phosphorylated state — blocks intermolecular interactions between the tail and the FERM domain[116,117]. Conversely, a mutation — S518A — that blocks phosphorylation at this site promotes these interactions. Experiments have shown that the hypophosphorylated, presumably folded, form of merlin interacts with the cytoplasmic tail of CD44, indicating that the effects of merlin on proliferation might be mediated through this interaction[118]. Taken together, these results indicate that it is probably the closed, dephosphorylated form of merlin that interacts with CD44 (thereby inhibiting proliferation), and therefore that this is the active form of the protein. Although it is possible that the merlin protein functions in a fundamentally different way in *Drosophila* compared with humans, this seems unlikely given the high degree of sequence conservation between these proteins and the observation that a transgene that carries the wild-type human *NF2* gene completely rescues the lethality of *Drosophila* merlin mutations[104]. Further tests, including *in vivo*

COILED-COIL DOMAINS
A protein domain that forms a bundle of two or three α-helices. Whereas short coiled-coil domains are involved in protein interactions, long coiled-coil domains that form long rods occur in structural or motor proteins.

CRE/*LOX*P
A site-specific recombination system that is derived from the *Escherichia coli* bacteriophage P1. Two short DNA sequences (*lox*P sites) are engineered to flank the target DNA. Activation of the Cre recombinase enzyme catalyses recombination between the *lox*P sites, which leads to the excision of the intervening sequence.

Box 3 | Animal models for the role of merlin in tumour suppression

Animal models for the Neurofibromatosis type 2 (NF2) disease have been developed using the mouse and the fruitfly, *Drosophila melanogaster*. In both systems, animals that are homozygous for loss-of-function mutations are not viable, which indicates that merlin has essential functions[23,24]. However, unlike humans, mice that are heterozygous for an *Nf2* mutation fail to develop the schwannomas and other tumours that characterize the NF2 disease. Instead, these mice develop cancers such as osteosarcomas, fibrosarcomas and hepatocellular carcinomas that are unusually metastatic in character[125]. This result indicates that *NF2* mutations might have a role in many cancers and could also alter the adhesive or motile properties of cells, possibly by altering cytoskeletal functions.

Why don't mice develop tumours that are characteristic of the human NF2 disease? Recent studies indicate that this is not due to a fundamental difference in the function of merlin in the mouse, but rather due to differences in the probability of loss of the wild-type allele in the crucial tissue — the Schwann cells. Giovannini and colleagues[126] have shown that mice that have a conditional *NF2* allele that can be specifically inactivated in Schwann cells using the CRE/*LOXP* system show Schwann-cell HYPERPLASIA and schwannomas that are derived from the mutant cells. More recently, they have shown that merlin inactivation in another glial cell type, the astrocyte, results in the formation of meningiomas[127]. These studies indicate that merlin function is well conserved between mice and humans, and therefore that the mouse is an effective model for the NF2 disease.

Are *Drosophila* also a good model for NF2 function? On the surface perhaps not, given that they lack the Schwann cells that are the focus of the human disease. However, analogous experiments in *Drosophila* using loss-of-function merlin mutations and the FLP/FRT system to generate somatic mosaic 'clones' of mutant epithelial cells have shown that just as in mammals, merlin-deficient *Drosophila* cells show hyperplasia[104]. So, merlin also has a tumour-suppressive function in *Drosophila*.

The real value of these animal models is the potential to create many molecular genetic tools with which to analyse the functions of genes and the proteins that they encode. In both systems, studies have been initiated to identify other genes that can modify phenotypes that are associated with merlin mutations. In the mouse, mutations in the p53 tumour suppressor are synergistic with *NF2* mutations[125], and in *Drosophila*, another protein 4.1 superfamily member, *expanded*, interacts both genetically and biochemically with merlin[128]. Transgenic methodologies in *Drosophila* have allowed an extensive structure–function analysis of merlin, whereas the ability to readily isolate mutant cells in primary culture from *Nf2*-deficient mice provides a powerful tool for analysis of the cellular effects of alterations in merlin function. So, animal models for NF2 disease have provided a key tool for understanding merlin functions, and in the future should have an important role in the development of therapeutic strategies.

functional analysis in transgenic mice, will probably be required to clarify this issue.

The tumour-suppressive role of merlin. Although mutational analysis in humans, mice and *Drosophila* all clearly indicate that merlin is important in the regulation of cellular proliferation (BOX 3), the mechanism by which this occurs is still unclear. Two recent studies shed some light on this question. Morrison *et al.*[118] have proposed that contact inhibition of cell proliferation is mediated through interactions between merlin and CD44. Their data indicate that in response to ligand binding, which occurs only at high cell density, CD44 mediates dephosphorylation and activation of merlin function, and that this thereby restricts cellular proliferation. Although the mechanism by which this occurs is unclear, Shaw *et al.*[117] have proposed that merlin functions to negatively regulate Rac, a Rho-FAMILY-GTPase member. Previous studies have shown that Rac activity is necessary for malignant transformation by activated forms of the related GTPase Ras, and that activated forms of Rac have oncogenic properties. In addition, increased Rac activity has been associated with increased cellular motility and might therefore affect metastatic potential[118]. Taken together, these studies indicate that merlin might restrict proliferation by regulating Rac pathway activity, and is itself regulated through the transmembrane protein CD44. What is still unclear is whether this is the only mechanism by which merlin regulates proliferation. Other studies have indicated that merlin interacts with other transmembrane proteins, and has other binding partners, such as HRS[111] that might regulate proliferation through other pathways. In addition, a recent study[119] in *Drosophila* has identified negative regulators of both the epidermal-growth-factor and transforming-growth-factor-β pathways as genetic modifiers of a dominant-negative merlin phenotype, which indicates that merlin might also negatively regulate these pathways.

HYPERPLASIA
An increase in the number of cells in a tissue or organ without gross morphological changes.

FLP/FLT
Flp encodes a recombinase that catalyses site-specific recombination between sites called Flp recognition targets (FRT). The Flp/FRT system has been successfully applied as a site-specific recombination system.

Rho FAMILY GTPases
Ras-related GTPases that are involved in controlling the organization of actin.

Perspectives

Since the discovery of ezrin as a component of the intestinal-brush border, work on the ERM proteins has concentrated primarily on their possible structural functions that link the plasma membrane to underlying cytoskeletal components. However, more recent studies, in particular the discovery that the *NF2* tumour-suppressor gene encodes the highly related protein merlin, have led to increasing interest in other possible roles, particularly in regulating signalling processes. The ability of ERM proteins to interact with transmembrane proteins, PDZ-domain-containing proteins and the cytoskeleton in a highly regulated fashion strongly indicates that they might have a role in organizing signalling complexes that function to regulate cytoskeletal assembly, membrane-protein function and membrane transport. One challenge for future research is to further explore this relatively poorly understood aspect of ERM function. Beyond this, we are still far from understanding how ERM proteins participate in the assembly of complex cellular structures, such as microvilli in epithelial cells or the leading edge of migratory cells. Genetic analysis of ERM function, particularly in non-mammalian model systems in which redundancy between the highly related ERM proteins is not a problem, should be particularly useful in determining the cellular functions of ERM proteins. Finally, a key area of future research will be to examine the relationship between ERM proteins and their close relative, merlin. Despite the high degree of structural similarity that is conserved from *Drosophila* to humans, we know very little about functional interactions between these proteins at present. These areas of research should have important implications for understanding epithelial polarity, cytoskeletal assembly, cellular migration and tumour suppression.

1. Sato, N. *et al.* A gene family consisting of ezrin, radixin and moesin. Its specific localization at actin filament/plasma membrane association sites. *J. Cell Sci.* **103**, 131–143 (1992).
2. Bretscher, A. Purification of an 80,000-dalton protein that is a component of the isolated microvillus cytoskeleton, and its localization in nonmuscle cells. *J. Cell Biol.* **97**, 425–432 (1983).
This paper reports on the original identification of ezrin as a component of microvillar cytoskeletons, its purification and localization to cell-surface microvilli of cultured cells.
3. Pakkanen, R., Hedman, K., Turunen, O., Wahlstrom, T. & Vaheri, A. Microvillus-specific M_r 75,000 plasma membrane protein of human choriocarcinoma cells. *J. Histochem. Cytochem.* **35**, 809–816 (1987).
4. Gould, K. L., Cooper, J. A., Bretscher, A. & Hunter, T. The protein-tyrosine kinase substrate, p81, is homologous to a chicken microvillar core protein. *J. Cell Biol.* **102**, 660–669 (1986).
5. Berryman, M., Franck, Z. & Bretscher, A. Ezrin is concentrated in the apical microvilli of a wide variety of epithelial cells whereas moesin is found primarily in endothelial cells. *J. Cell Sci.* **105**, 1025–1043 (1993).
6. Tsukita, S., Hieda, Y. & Tsukita, S. A new 82-kD barbed end-capping protein (radixin) localized in the cell-to-cell adherens junction: purification and characterization. *J. Cell Biol.* **108**, 2369–2382 (1989).
The first description of radixin.
7. Amieva, M. R., Wilgenbus, K. K. & Furthmayr, H. Radixin is a component of hepatocyte microvilli *in situ*. *Exp. Cell Res.* **210**, 140–144 (1994).
8. Lankes, W., Griesmacher, A., Grunwald, J., Schwartz-Albiez, R. & Keller, R. A heparin-binding protein involved in inhibition of smooth-muscle cell proliferation. *Biochem. J.* **251**, 831–842 (1988).
9. Gould, K. L., Bretscher, A., Esch, F. S. & Hunter, T. cDNA cloning and sequencing of the protein-tyrosine kinase substrate, ezrin, reveals homology to band 4.1. *EMBO J.* **8**, 4133–4142 (1989).
10. Lankes, W. T. & Furthmayr, H. Moesin: a member of the protein 4.1–talin–ezrin family of proteins. *Proc. Natl Acad. Sci. USA* **88**, 8297–8301 (1991).
This study showed that moesin is closely related to ezrin.
11. Funayama, N., Nagafuchi, A., Sato, N., Tsukita, S. & Tsukita, S. Radixin is a novel member of the band 4.1 family. *J. Cell Biol.* **115**, 1039–1048 (1991).
12. Trofatter, J. A. *et al.* A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. *Cell* **75**, 826 (1993).
13. Rouleau, G. A. *et al.* Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. *Nature* **363**, 515–521 (1993).
Together with reference 12, this study showed that the complementary DNA that encodes the product of the *Neurofibromatosis 2* tumour-suppressor gene is related to the ERM family.
14. Gusella, J. F., Ramesh, V., MacCollin, M. & Jacoby, L. B. Merlin: the neurofibromatosis 2 tumor suppressor. *Biochim. Biophys. Acta* **1423**, M29–M36 (1999).
15. Bretscher, A., Chamber, C., Nguyen, R. & Reczek, D. ERM-merlin and EBP50 protein families in plasma membrane organization and function. *Annu. Rev. Cell Dev. Biol.* **16**, 113–143 (2000).
16. Mangeat, P., Roy, C. & Martin, M. ERM proteins in cell adhesion and membrane dynamics. *Trends Cell Biol.* **9**, 187–192 (1999).
17. Tsukita, S. & Yonemura, S. Cortical actin organization: lessons from ERM (ezrin/radixin/moesin) proteins. *J. Biol. Chem.* **274**, 34507–34510 (1999).
18. Doi, Y. *et al.* Normal development of mice and unimpaired cell adhesion/cell motility/actin-based cytoskeleton without compensatory up-regulation of ezrin or radixin in moesin gene knockout. *J. Biol. Chem.* **274**, 2315–2321 (1999).
19. Takeuchi, K. *et al.* Perturbation of cell adhesion and microvilli formation by antisense oligonucleotides to ERM family members. *J. Cell Biol.* **125**, 1371–1384 (1994).
Using antisense oligonucleotides to suppress the expression of ERM proteins, this study showed that ERM proteins are necessary for the presence of microvilli on cultured cells.
20. Allenspach, E. J. *et al.* ERM-dependent movement of CD43 defines a novel protein complex distal to the immunological synapse. *Immunity* **15**, 739–750 (2001).
21. Kaul, S. C. *et al.* Identification of a 55-kDa ezrin-related protein that induces cytoskeletal changes and localizes to the nucleolus. *Exp. Cell Res.* **250**, 51–61 (1999).
22. Crepaldi, T., Gautreau, A., Comoglio, P. M., Louvard, D. & Arpin, M. Ezrin is an effector of hepatocyte growth factor-mediated migration and morphogenesis in epithelial cells. *J. Cell Biol.* **138**, 423–434 (1997).
23. McClatchey, A. I., Saotome, I., Ramesh, V., Gusella, J. F. & Jacks, T. The *Nf2* tumor suppressor gene product is essential for extraembryonic development immediately prior to gastrulation. *Genes Dev.* **11**, 1253–1265 (1997).
This paper reports the first mouse knockout of the *Nf2* gene, which shows that merlin is essential for early development.
24. Fehon, R. G., Oren, T., LaJeunesse, D. R., Melby, T. E. & McCartney, B. M. Isolation of mutations in the *Drosophila* homologues of the human *Neurofibromatosis 2* and yeast *CDC42* genes using a simple and efficient reverse-genetic method. *Genetics* **146**, 245–252 (1997).
25. Gusella, J. F., Ramesh, V., MacCollin, M. & Jacoby, L. B. Neurofibromatosis 2: loss of merlin's protective spell. *Curr. Opin. Genet. Dev.* **6**, 87–92 (1996).
26. Gary, R. & Bretscher, A. Heterotypic and homotypic associations between ezrin and moesin, two putative membrane-cytoskeletal linking proteins. *Proc. Natl Acad. Sci. USA* **90**, 10846–10850 (1993).
27. Gary, R. & Bretscher, A. Ezrin self-association involves binding of an N-terminal domain to a normally masked C-terminal domain that includes the F-actin binding site. *Mol. Biol. Cell* **6**, 1061–1075 (1995).
The intramolecular FERM–C-ERMAD interaction is described and mapped, and the idea that ERM proteins are subject to conformational regulation is proposed.
28. Berryman, M., Gary, R. & Bretscher, A. Ezrin oligomers are major cytoskeletal components of placental microvilli: a proposal for their involvement in cortical morphogenesis. *J. Cell Biol.* **131**, 1231–1242 (1995).
29. Bretscher, A., Gary, R. & Berryman, M. Soluble ezrin purified from placenta exists as stable monomers and elongated dimers with masked C-terminal ezrin–radixin–moesin association domains. *Biochemistry* **34**, 16830–16837 (1995).
30. Magendantz, M., Henry, M. D., Lander, A. & Solomon, F. Interdomain interactions of radixin *in vitro*. *J. Biol. Chem.* **270**, 25324–25327 (1995).
31. Martin, M. *et al.* Ezrin NH_2-terminal domain inhibits the cell extension activity of the COOH-terminal domain. *J. Cell. Biol.* **128**, 1081–1093 (1995).
32. Henry, M. D., Gonzalez Agosti, C. & Solomon, F. Molecular dissection of radixin: distinct and interdependent functions of the amino- and carboxy-terminal domains. *J. Cell Biol.* **129**, 1007–1022 (1995).
33. Reczek, D. & Bretscher, A. The carboxyl-terminal region of EBP50 binds to a site in the amino-terminal domain of ezrin that is masked in the dormant molecule. *J. Biol. Chem.* **273**, 18452–18458 (1998).
This paper used biochemical studies to prove that the binding site of EBP50 on the FERM domain is masked by the FERM–C-ERMAD interaction in dormant ezrin, which validates the conformational-regulation hypothesis.
34. Takahashi, K. *et al.* Direct interaction of the Rho GDP dissociation inhibitor with ezrin/radixin/moesin initiates the activation of the Rho small G protein. *J. Biol. Chem.* **272**, 23371–23375 (1997).
35. Ishikawa, H. *et al.* Structural conversion between open and closed forms of radixin: low-angle shadowing electron microscopy. *J. Mol. Biol.* **310**, 973–978 (2001).
36. Gautreau, A., Louvard, D. & Arpin, M. Morphogenic effects of ezrin require a phosphorylation-induced transition from oligomers to monomers at the plasma membrane. *J. Cell Biol.* **150**, 193–203 (2000).
37. Nakamura, F., Amieva, M. R. & Furthmayr, H. Phosphorylation of threonine 558 in the carboxyl-terminal actin-binding domain of moesin by thrombin activation of human platelets. *J. Biol. Chem.* **270**, 31377–31385 (1995).
38. Matsui, T. *et al.* Rho-kinase phosphorylates COOH-terminal threonines of ezrin/radixin/moesin (ERM) proteins and regulates their head-to-tail association. *J. Cell Biol.* **140**, 647–657 (1998).
The *in vitro* demonstration that Rho-kinase can phosphorylate a specific threonine in the carboxy-terminal region of ERM proteins, which reduces the FERM–C-ERMAD interaction, but has no effect on the F-actin-binding site.
39. Oshiro, N., Fukata, Y. & Kaibuchi, K. Phosphorylation of moesin by Rho-associated kinase (Rho-kinase) plays a crucial role in the formation of microvilli-like structures. *J. Biol. Chem.* **273**, 34663–34666 (1998).
40. Hayashi, K., Yonemura, S., Matsui, T., Tsukita, S. & Tsukita, S. Immunofluorescence detection of ezrin/radixin/moesin (ERM) proteins with their carboxyl-terminal threonine phosphorylated in cultured cells and tissues. *J. Cell Sci.* **112**, 1149–1158 (1999).
41. Tran Quang, C., Gautreau, A., Arpin, M. & Treisman, R. Ezrin function is required for ROCK-mediated fibroblast transformation by the *net* and *dbl* oncogenes. *EMBO J.* **19**, 4565–4576 (2000).
42. Ng, T. *et al.* Ezrin is a downstream effector of trafficking PKC–integrin complexes involved in the control of cell motility. *EMBO J.* **20**, 2723–2741 (2001).
43. Pietromonaco, S. F., Simons, P. C., Altman, A. & Elias, L. Protein kinase C-θ phosphorylation of moesin in the actin-binding sequence. *J. Biol. Chem.* **273**, 7594–7603 (1998).
44. Simons, P. C., Pietromonaco, S. F., Reczek, D., Bretscher, A. & Elias, L. C-terminal threonine phosphorylation activates ERM proteins to link the cell's cortical lipid bilayer to the cytoskeleton. *Biochem. Biophys. Res. Commun.* **253**, 561–565 (1998).
45. Yonemura, S., Matsui, T. & Tsukita, S. Rho-dependent and-independent activation mechanisms of ezrin/radixin/moesin proteins: an essential role for polyphosphoinositides *in vivo*. *J. Cell Sci.* **115**, 2569–2580 (2002).
46. Hirao, M. *et al.* Regulation mechanism of ERM (ezrin/radixin/moesin) protein/plasma membrane association: possible involvement of phosphatidylinositol turnover and Rho-dependent signaling pathway. *J. Cell Biol.* **135**, 37–51 (1996).
This study proposed roles for PtdIns(4,5)P_2 and Rho-signalling pathways in ERM protein regulation.
47. Yonemura, S. *et al.* Ezrin/radixin/moesin (ERM) proteins bind to a positively charged amino acid cluster in the juxta-membrane cytoplasmic domain of CD44, CD43, and ICAM-2. *J. Cell Biol.* **140**, 885–895 (1998).
Together with reference 68, this report provided the first identification of ERM-binding regions in the cytoplasmic tails of membrane proteins.
48. Nakamura, F., Huang, L., Pestonjamasp, K., Luna, E. J. & Furthmayr, H. Regulation of F-actin binding to platelet moesin *in vitro* by both phosphorylation of threonine 558 and polyphosphatidylinositides. *Mol. Biol. Cell* **10**, 2669–2685 (1999).
49. Niggli, V., Andreoli, C., Roy, C. & Mangeat, P. Identification of a phosphatidylinositol-4,5-bisphosphate-binding domain in the N-terminal region of ezrin. *FEBS Lett.* **376**, 172–176 (1995).
50. Barret, C., Roy, C., Montcourrier, P., Mangeat, P. & Niggli, V. Mutagenesis of the phosphatidylinositol 4,5-bisphosphate (PIP_2) binding site in the NH_2-terminal domain of ezrin correlates with its altered cellular distribution. *J. Cell Biol.* **151**, 1067–1080 (2000).
51. Pearson, M., Reczek, D., Bretscher, A. & Karplus, P. Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain. *Cell* **101**, 259–270 (2000).
This study reported the first structure of a FERM domain, as well as the FERM–C-ERMAD complex.
52. Hamada, K., Shimizu, T., Matsui, T., Tsukita, S. & Hakoshima, T. Structural basis of the membrane-targeting and unmasking mechanisms of the radixin FERM domain. *EMBO J.* **19**, 4449–4462 (2000).
This study reported the first structure of the free (and presumably active) FERM domain and, by comparison with the structure of Pearson *et al.* (reference 51), described the structural changes that are induced after C-ERMAD release.
53. Edwards, S. D. & Keep, N. H. The 2.7 Å crystal structure of the activated FERM domain of moesin: an analysis of structural changes on activation. *Biochemistry* **40**, 7061–7068 (2001).
54. Zhou, Y. J. *et al.* Unexpected effects of FERM domain mutations on catalytic activity of Jak3: structural implication for Janus kinases. *Mol. Cell* **8**, 959–969 (2001).
55. Gu, M. & Majerus, P. W. The properties of the protein tyrosine phosphatase PTPMEG. *J. Biol. Chem.* **271**, 27751–27759 (1996).
56. Johnson, R. P. & Craig, S. W. F-actin binding site masked by the intramolecular association of vinculin head and tail domains. *Nature* **373**, 261–264 (1995).
57. Johnson, R. P. & Craig, S. W. Actin activates a cryptic dimerization potential of the vinculin tail domain. *J. Biol. Chem.* **275**, 95–105 (2000).
58. Rohatgi, R. *et al.* The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* **97**, 221–231 (1999).
59. Alberts, A. S. Identification of a carboxyl-terminal diaphanous-related formin homology protein autoregulatory domain. *J. Biol. Chem.* **276**, 2824–2830 (2001).
60. Bretscher, A. Rapid phosphorylation and reorganization of ezrin and spectrin accompany morphological changes induced in A-431 cells by epidermal growth factor. *J. Cell*

Biol. **108**, 921–930 (1989).
61. Turunen, O., Wahlstrom, T. & Vaheri, A. Ezrin has a COOH-terminal actin-binding site that is conserved in the ezrin protein family. *J. Cell Biol.* **126**, 1445–1453 (1994).
This study identified the F-actin-binding site in the tail of ezrin.
62. Pestonjamasp, K. *et al.* Moesin, ezrin, and p205 are actin-binding proteins associated with neutrophil plasma membranes. *Mol. Biol. Cell* **6**, 247–259 (1995).
63. Berryman, M. & Bretscher, A. Identification of a novel member of the chloride intracellular channel gene family (*CLIC5*) that associates with the actin cytoskeleton of placental microvilli. *Mol. Biol. Cell* **11**, 1509–1521 (2000).
64. Roy, C., Martin, M. & Mangeat, P. A dual involvement of the amino-terminal domain of ezrin in F- and G-actin binding. *J. Biol. Chem.* **272**, 20088–20095 (1997).
65. Martin, M., Roy, C., Montcourrier, P., Sahuquet, A. & Mangeat, P. Three determinants in ezrin are responsible for cell extension activity. *Mol. Biol. Cell* **8**, 1543–1557 (1997).
66. Marchesi, V. T. Stabilizing infrastructure of cell membranes. *Annu. Rev. Cell Biol.* **1**, 531–561 (1985).
67. Tsukita, S. *et al.* ERM family members as molecular linkers between the cell surface glycoprotein CD44 and actin-based cytoskeletons. *J. Cell. Biol.* **126**, 391–401 (1994).
68. Legg, J. W. & Isacke, C. M. Identification and functional analysis of the ezrin-binding site in the hyaluronan receptor, CD44. *Curr. Biol.* **8**, 705–708 (1998).
69. Legg, J. W., Lewis, C. A., Parsons, M., Ng, T. & Isacke, C. M. A novel PKC-regulated mechanism controls CD44 ezrin association and directional cell motility. *Nature Cell Biol.* **4**, 399–407 (2002).
70. Heiska, L. *et al.* Association of ezrin with intercellular adhesion molecule-1 and-2 (ICAM-1 and ICAM-2). Regulation by phosphatidylinositol 4,5-bisphosphate. *J. Biol. Chem.* **273**, 21893–21900 (1998).
71. Helander, T. S. *et al.* ICAM-2 redistributed by ezrin as a target for killer cells. *Nature* **382**, 265–268 (1996).
The first evidence for a biological function of ezrin.
72. Shaw, A. S. FERMing up the synapse. *Immunity* **15**, 683–686 (2001).
73. Delon, J., Kaibuchi, K. & Germain, R. N. Exclusion of CD43 from the immunological synapse is mediated by phosphorylation-regulated relocation of the cytoskeletal adaptor moesin. *Immunity* **15**, 691–701 (2001).
74. Roumier, A. *et al.* The membrane-microfilament linker ezrin is involved in the formation of the immunological synapse and in T cell activation. *Immunity* **15**, 715–728 (2001).
75. Denker, S. P., Huang, D. C., Orlowski, J., Furthmayr, H. & Barber, D. L. Direct binding of the Na–H exchanger NHE1 to ERM proteins regulates the cortical cytoskeleton and cell shape independently of H^+ translocation. *Mol. Cell* **6**, 1425–1436 (2000).
76. Yun, C. H., Lamprecht, G., Forster, D. V. & Sidor, A. NHE3 kinase A regulatory protein E3KARP binds the epithelial brush border Na^+/H^+ exchanger NHE3 and the cytoskeletal protein ezrin. *J. Biol. Chem.* **273**, 25856–25863 (1998).
77. Reczek, D. & Bretscher, A. Identification of EPI64, a TBC/RabGAP domain-containing microvillar protein that binds to the first PDZ domain of EBP50 and E3KARP. *J. Cell Biol.* **153**, 191–206 (2001).
78. Weinman, E. J., Minkoff, C. & Shenolikar, S. Signal complex regulation of renal transport proteins: NHERF and regulation of NHE3 by PKA. *Am. J. Physiol. Renal Physiol.* **279**, F393–F399 (2000).
79. Weinman, E. J. New functions for the NHERF family of proteins. *J. Clin. Invest.* **108**, 185–186 (2001).
80. Weinman, E. J. & Shenolikar, S. The Na–H exchanger regulatory factor. *Exp. Nephrol.* **5**, 449–452 (1997).
81. Weinman, E. J., Steplock, D., Wade, J. B. & Shenolikar, S. Ezrin binding domain-deficient NHERF attenuates cAMP-mediated inhibition of Na^+/H^+ exchange in OK cells. *Am. J. Physiol. Renal Physiol.* **281**, F374–F380 (2001).
82. Dransfield, D. T. *et al.* Ezrin is a cyclic AMP-dependent protein kinase anchoring protein. *EMBO J.* **16**, 35–43 (1997).
83. Wang, S., Raab, R. W., Schatz, P. J., Guggino, W. B. & Li, M. Peptide binding consensus of the NHE-RF–PDZ1 domain matches the C-terminal sequence of cystic fibrosis transmembrane conductance regulator (CFTR). *FEBS Lett.* **427**, 103–108 (1998).
84. Short, D. B. *et al.* An apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton. *J. Biol. Chem.* **273**, 19797–19801 (1998).
85. Hall, R. A. *et al.* A C-terminal motif found in the β2-adrenergic receptor, P2Y1 receptor and cystic fibrosis transmembrane conductance regulator determines binding to the Na^+/H^+ exchanger regulatory factor family of PDZ proteins. *Proc. Natl Acad. Sci. USA* **95**, 8496–8501 (1998).
86. Hall, R. A. *et al.* The β2-adrenergic receptor interacts with the Na^+/H^+-exchanger regulatory factor to control Na^+/H^+ exchange. *Nature* **392**, 626–630 (1998).
87. Cao, T. T., Deacon, H. W., Reczek, D., Bretscher, A. & von Zastrow, M. A kinase-regulated PDZ-domain interaction controls endocytic sorting of the β2-adrenergic receptor. *Nature* **401**, 286–290 (1999).
88. Yun, C. H. *et al.* cAMP-mediated inhibition of the epithelial brush border Na^+/H^+ exchanger, NHE3, requires an associated regulatory protein. *Proc. Natl Acad. Sci. USA* **94**, 3010–3015 (1997).
89. Maudsley, S. *et al.* Platelet-derived growth factor receptor association with Na^+/H^+ exchanger regulatory factor potentiates receptor activity. *Mol. Cell. Biol.* **20**, 8352–8363 (2000).
90. Takeda, T., McQuistan, T., Orlando, R. A. & Farquhar, M. G. Loss of glomerular foot processes is associated with uncoupling of podocalyxin from the actin cytoskeleton. *J. Clin. Invest.* **108**, 289–301 (2001).
91. Matsui, T., Yonemura, S., Tsukita, S. & Tsukita, S. Activation of ERM proteins *in vivo* by rho involves phosphatidylinositol 4-phosphate 5-kinase and not ROCK kinases. *Curr. Biol.* **9**, 1259–1262 (1999).
92. Fukata, Y. *et al.* Association of the myosin-binding subunit of myosin phosphatase and moesin: dual regulation of moesin phosphorylation by Rho-associated kinase and myosin phosphatase. *J. Cell Biol.* **141**, 409–418 (1998).
93. Shaw, R. J., Henry, M., Solomon, F. & Jacks, T. RhoA-dependent phosphorylation and relocalization of ERM proteins into apical membrane/actin protrusions in fibroblasts. *Mol. Biol. Cell* **9**, 403–419 (1998).
94. Kotani, H., Takaishi, K., Sasaki, T. & Takai, Y. Rho regulates association of both the ERM family and vinculin with the plasma membrane in MDCK cells. *Oncogene* **14**, 1705–1713 (1997).
95. Mackay, D. J., Esch, F., Furthmayr, H. & Hall, A. Rho- and Rac-dependent assembly of focal adhesion complexes and actin filaments in permeabilized fibroblasts: an essential role for ezrin/radixin/moesin proteins. *J. Cell Biol.* **138**, 927–938 (1997).
96. Lamb, R. F. *et al.* The TSC1 tumour suppressor hamartin regulates cell adhesion through ERM proteins and the GTPase Rho. *Nature Cell Biol.* **2**, 281–287 (2000).
97. Shimizu, T. *et al.* Structural basis for Neurofibromatosis type 2. Crystal structure of the merlin FERM domain. *J. Biol. Chem.* **277**, 10332–10336 (2002).
98. Kang, B. S., Cooper, D. R., Devedjiev, Y., Derewenda, U. & Derewenda, Z. S. The structure of the FERM domain of merlin, the neurofibromatosis type 2 gene product. *Acta Crystallogr. D Biol. Crystallogr.* **58**, 381–391 (2002).
99. McCartney, B. M. & Fehon, R. G. Distinct cellular and subcellular patterns of expression imply distinct functions for the *Drosophila* homologues of moesin and the neurofibromatosis 2 tumor suppressor, merlin. *J. Cell Biol.* **133**, 843–852 (1996).
100. Xu, H. M. & Gutmann, D. H. Merlin differentially associates with the microtubule and actin cytoskeleton. *J. Neurosci. Res.* **51**, 403–415 (1998).
101. James, M. F., Manchanda, N., Gonzalez-Agosti, C., Hartwig, J. H. & Ramesh, V. The neurofibromatosis 2 protein product merlin selectively binds F-actin but not G-actin, and stabilizes the filaments through a lateral association. *Biochem. J.* **356**, 377–386 (2001).
102. Brault, E. *et al.* Normal membrane localization and actin association of the NF2 tumor suppressor protein are dependent on folding of its N-terminal domain. *J. Cell Sci.* **114**, 1901–1912 (2001).
103. Scherer, S. S., Xu, T., Crino, P., Arroyo, E. J. & Gutmann, D. H. Ezrin, radixin, and moesin are components of Schwann cell microvilli. *J. Neurosci. Res.* **65**, 150–164 (2001).
104. LaJeunesse, D. R., McCartney, B. M. & Fehon, R. G. Structural analysis of *Drosophila* merlin reveals functional domains important for growth control and subcellular localization. *J. Cell Biol.* **141**, 1589–1599 (1998).
This study showed that merlin regulates cell proliferation in *Drosophila*, and the FERM domain provides all the essential functions of *Drosophila* merlin.
105. Stokowski, R. P. & Cox, D. R. Functional analysis of the neurofibromatosis type 2 protein by means of disease-causing point mutations. *Am. J. Hum. Genet.* **66**, 873–891 (2000).
106. Meng, J. J. *et al.* Interaction between two isoforms of the NF2 tumor suppressor protein, merlin, and between merlin and ezrin, suggests modulation of ERM proteins by merlin. *J. Neurosci. Res.* **62**, 491–502 (2000).
107. Gronholm, M. *et al.* Homotypic and heterotypic interaction of the neurofibromatosis 2 tumor suppressor protein merlin and the ERM protein ezrin. *J. Cell Sci.* **112**, 895–904 (1999).
108. Gonzalez-Agosti, C., Wiederhold, T., Herndon, M. E., Gusella, J. & Ramesh, V. Interdomain interaction of merlin isoforms and its influence on intermolecular binding to NHE-RF. *J. Biol. Chem.* **274**, 34438–34442 (1999).
109. Sherman, L. *et al.* Interdomain binding mediates tumor growth suppression by the NF2 gene product. *Oncogene* **15**, 2505–2509 (1997).
110. Nguyen, R., Reczek, D. & Bretscher, A. Hierarchy of merlin and ezrin N- and C-terminal domain interactions in homo- and heterotypic associations and their relationship to binding of scaffolding proteins EBP50 and E3KARP. *J. Biol. Chem.* **276**, 7621–7629 (2001).
111. Gutmann, D. H. *et al.* The NF2 interactor, hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), associates with merlin in the 'open' conformation and suppresses cell growth and motility. *Hum. Mol. Genet.* **10**, 825–834 (2001).
112. Maeda, M., Matsui, T., Imamura, M., Tsukita, S. & Tsukita, S. Expression level, subcellular distribution and Rho-GDI binding affinity of merlin in comparison with ezrin/radixin/moesin proteins. *Oncogene* **18**, 4788–4797 (1999).
113. Scoles, D. R. *et al.* Neurofibromatosis 2 tumour suppressor schwannomin interacts with βII-spectrin. *Nature Genet.* **18**, 354–359 (1998).
114. Xiao, G. H., Beeser, A., Chernoff, J. & Testa, J. R. p21-activated kinase links Rac/Cdc42 signaling to Merlin. *J. Biol. Chem.* **21**, 21 (2001).
115. Kissil, J. L., Johnson, K. C., Eckman, M. S. & Jacks, T. Merlin phosphorylation by p21-activated kinase 2 and effects of phosphorylation on merlin localization. *J. Biol. Chem.* **277**, 10394–10399 (2002).
116. Shaw, R. J., McClatchey, A. I. & Jacks, T. Regulation of the neurofibromatosis type 2 tumor suppressor protein, merlin, by adhesion and growth arrest stimuli. *J. Biol. Chem.* **273**, 7757–7764 (1998).
117. Shaw, R. J. *et al.* The Nf2 tumor suppressor, merlin, functions in Rac-dependent signaling. *Dev. Cell* **1**, 63–72 (2001).
This study showed that merlin functions downstream of Rac in a signalling pathway.
118. Morrison, H. *et al.* The *NF2* tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. *Genes Dev.* **15**, 968–980 (2001).
Merlin is reported to confer its tumour-suppressing function through interaction with the cytoplasmic tail of CD44 in response to elevated levels of hyaluronate, the ligand for CD44.
119. LaJeunesse, D. R., McCartney, B. M. & Fehon, R. G. A systematic screen for dominant second-site modifiers of Merlin/NF2 phenotypes reveals an interaction with blistered/DSRF and scribbler. *Genetics* **158**, 667–679 (2001).
120. Chishti, A. H. *et al.* The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane. *Trends Biochem. Sci.* **23**, 281–282 (1998).
121. Thompson, J. D., Higgins, D. G. & Gibson, T. J. CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680 (1994).
122. Girault, J. A., Labesse, G., Mornon, J. P. & Callebaut, I. Janus kinases and focal adhesion kinases play in the 4.1 band: a superfamily of band 4.1 domains important for cell structure and signal transduction. *Mol. Med.* **4**, 751–769 (1998).
123. Edwards, K., Davis, T., Marcey, D., Kurihara, J. & Yamamoto, D. Comparative analysis of the Band 4.1/ezrin-related protein tyrosine phosphatase Pez from two *Drosophila* species: implications for structure and function. *Gene* **275**, 195–205 (2001).
124. Andersen, J. N. *et al.* Structural and evolutionary relationships among protein tyrosine phosphatase domains. *Mol. Cell. Biol.* **21**, 7117–7136 (2001).
125. McClatchey, A. I. *et al.* Mice heterozygous for a mutation at the *Nf2* tumor suppressor locus develop a range of highly metastatic tumors. *Genes Dev.* **12**, 1121–1133 (1998).
126. Giovannini, M. *et al.* Schwann cell hyperplasia and tumors in transgenic mice expressing a naturally occurring mutant NF2 protein. *Genes Dev.* **13**, 978–986 (1999).
127. Kalamarides, M. *et al. Nf2* gene inactivation in arachnoidal cells is rate-limiting for meningioma development in the mouse. *Genes Dev.* **16**, 1060–1065 (2002).
128. McCartney, B. M., Kulikauskas, R. M., LaJeunesse, D. R. & Fehon, R. G. The neurofibromatosis-2 homologue, merlin, and the tumor suppressor expanded function together in *Drosophila* to regulate cell proliferation and differentiation. *Development* **127**, 1315–1324 (2000).
129. Serrador, J. M. *et al.* CD43 interacts with moesin and ezrin and regulates its redistribution to the uropods of T lymphocytes at the cell–cell contacts. *Blood* **91**, 4632–4644 (1998).
130. Serrador, J. M. *et al.* Moesin interacts with the cytoplasmic region of intercellular adhesion molecule-3 and is redistributed to the uropod of T lymphocytes during cell polarization. *J. Cell Biol.* **138**, 1409–1423 (1997).

131. Ivetic, A., Deka, J., Ridley, A. & Ager, A. The cytoplasmic tail of L-selectin interacts with members of the ezrin–radixin–moesin (ERM) family of proteins: activation dependent binding of moesin but not ezrin. *J. Biol. Chem.* **8**, 8 (2001).
132. Reczek, D., Berryman, M. & Bretscher, A. Identification of EBP50: a PDZ-containing phosphoprotein that associates with members of the ezrin–radixin–moesin family. *J. Cell Biol.* **139**, 169–179 (1997).
The description of the first PDZ-containing apical scaffolding protein that binds the ERM family.
133. Bonilha, V. L. & Rodriguez-Boulan, E. Polarity and developmental regulation of two PDZ proteins in the retinal pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* **42**, 3274–3282 (2001).
134. Granes, F., Urena, J. M., Rocamora, N. & Vilaro, S. Ezrin links syndecan-2 to the cytoskeleton. *J. Cell Sci.* **113**, 1267–1276 (2000).
135. Takahashi, K. *et al.* Interaction of radixin with Rho small G protein GDP/GTP exchange protein Dbl. *Oncogene* **16**, 3279–3284 (1998).
136. Poullet, P. *et al.* Ezrin interacts with focal adhesion kinase and induces its activation independently of cell-matrix adhesion. *J. Biol. Chem.* **276**, 37686–37691 (2001).
137. Gautreau, A., Poullet, P., Louvard, D. & Arpin, M. Ezrin, a plasma membrane-microfilament linker, signals cell survival through the phosphatidylinositol 3-kinase/Akt pathway. *Proc. Natl Acad. Sci. USA* **96**, 7300–7305 (1999).
138. Parlato, S. *et al.* CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway. *EMBO J.* **19**, 5123–5134 (2000).
139. Mykkanen, O. M. *et al.* Characterization of human palladin, a microfilament-associated protein. *Mol. Biol. Cell* **12**, 3060–3073 (2001).

Acknowledgements
We thank D. Chambers for Figure 2a, and B. McCartney for Figure 2b–f. Work in the authors' laboratories was supported by grants from the NIH (A.B. and R.G.F.) and the US Army Neurofibromatosis Research Program (R.G.F.).

Online links

DATABASES
The following terms in this article are linked online to:
FlyBase: http://flybase.bio.indiana.edu/
expanded
Interpro: http://www.ebi.ac.uk/interpro/
ankyrin repeat region | myosin motor domain | PH | protein tyrosine phosphatase domain | RING finger | WW domain
OMIM: http://www.ncbi.nlm.nih.gov/Omim/
Neurofibromatosis type 2
Swiss-Prot: http://www.expasy.ch/
β-adrenergic receptor | cystic fibrosis transmembrane conductance regulator | CD44 | E3KARP | ezrin | hamartin | ICAM-2 | Jak3 | moesin | *Nf2* | NHE-1 | NHE-3 | radixin | RhoGDI | vinculin

FURTHER READING
The Biology Workbench (for CLUSTALW and DRAWTREE):
http://workbench.sdsc.edu
Access to this interactive links box is free online.