Protein 4.1 tumor suppressors: getting a FERM grip on growth regulation

Chun-Xiao Sun*, Victoria A. Robb* and David H. Gutmann‡

Department of Neurology, Washington University School of Medicine, St Louis, MO 63110, USA

*These authors contributed equally to this work

‡Author for correspondence (e-mail: gutmannd@neuro.wustl.edu)

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Summary

Members of the Protein 4.1 superfamily have highly conserved FERM domains that link cell surface glycoproteins to the actin cytoskeleton. Within this large and constantly expanding superfamily, at least five subgroups have been proposed. Two of these subgroups, the ERM and prototypic Protein 4.1 molecules, include proteins that function as tumor suppressors. The ERM subgroup member merlin/schwannomin is inactivated in the tumor-predisposition syndrome neurofibromatosis 2 (NF2), and the prototypic 4.1 subgroup member, Protein 4.1B, has been implicated in the molecular pathogenesis of breast, lung and brain cancers. This review focuses on what is known of mechanisms of action and critical protein interactions that may mediate the unique growth inhibitory signals of these two Protein 4.1 tumor suppressors. On the basis of insights derived from studying the *NF2* tumor suppressor, we propose a model for merlin growth regulation in which CD44 links growth signals from plasma membrane to the nucleus by interacting with ERM proteins and merlin.

Key words: Merlin, DAL-1, Tumor suppressor, Schwannomin

Introduction

Members of the Protein 4.1 superfamily are characterized by the presence of a conserved FERM (Four.1 protein, Ezrin, Radixin, Moesin) domain at the N-terminus of the molecule (Chishti et al., 1998) and, in many cases, a spectrin/actin binding domain (SABD). The name Protein 4.1 derives from the gel position of the founding member of the Protein 4.1 superfamily, Protein 4.1R, following 2D SDS polyacrylamide gel electrophoresis (Holzwarth et al., 1976). This protein, also known as erythrocyte band 4.1 protein, was originally identified as an abundant protein in human erythrocytes that localizes to the cytoskeleton and stabilizes red blood cell shape.

The Protein 4.1 superfamily has grown significantly since the identification of the original founding member, with more then 40 members identified to date. On the basis of protein sequence similarity, this superfamily can be classified into five subgroups: Protein 4.1 molecules, ERM proteins, talin-related molecules, PTPH (protein tyrosine phosphatases) proteins and NBL4 (novel band 4.1-like 4) (Takeuchi et al., 1994) proteins (Fig. 1). Talin is a 200 kDa protein concentrated at focal contacts, where it has been hypothesized to modulate binding of integrins to the cytoskeleton (Burridge and Connell, 1983). Unlike other Protein 4.1 molecules, PTPH and NBL4 proteins lack characterized actin-binding domains. The PTPH family includes at least three protein tyrosine phosphatases containing an N-terminal FERM domain and a C-terminal phosphatase domain (Gu et al., 1991). Localization of PTP-BL (protein-tyrosine phosphatase-BAS-like) to the apical side of epithelial cells requires the FERM domain (Cuppen et al., 1999). Another PTPH protein, PTP-FERM, is present in neuronal processes, where it localizes to the peri-membrane region through its FERM domain (Uchida et al., 2002). NBL4 proteins contain an N-terminal FERM domain and a unique non-homologous C-terminus (Takeuchi et al., 1994). A novel member of the NBL4 family (EHM2) has been implicated in melanoma tumor metastasis (Shimizu et al., 2000).

In addition to linking cell surface proteins to the actin cytoskeleton, members of the Protein 4.1 family have an additional function: the neurofibromatosis 2 (NF2) gene product, merlin/schwannomin, and Protein 4.1B/DAL-1 are negative growth regulators (tumor suppressors). This unique function of Protein 4.1 molecules is the focus of this article.

The Protein 4.1 and ERM subfamilies

The Protein 4.1 ERM molecules were originally characterized as structural components of the cell membrane. Their tissue distributions, subcellular localizations, and chromosomal assignments are summarized in Table 1. Although their tissue and subcellular distributions overlap, there are distinct differences between these related proteins that may explain their unique functions in specific tissues. For example, although merlin and the other three ERM proteins are all expressed in the rat sciatic nerve, there are significant differences in their subcellular distributions in vivo (Scherer and Gutmann, 1996; Scherer et al., 2001).

The Protein 4.1 family includes Protein 4.1R (erythrocyte), Protein 4.1G (general), Protein 4.1N (neuronal) and Protein 4.1B (brain). Each protein has a distinct expression pattern and is encoded by individual genes [Table 1 (Peters et al., 1998; Parra et al., 2000)]. This family of proteins is characterized by the presence of three highly conserved domains: an N-terminal FERM domain, a spectrin-actin-binding domain (SABD), and a C-terminal domain (CTD, Fig. 1). In addition to these

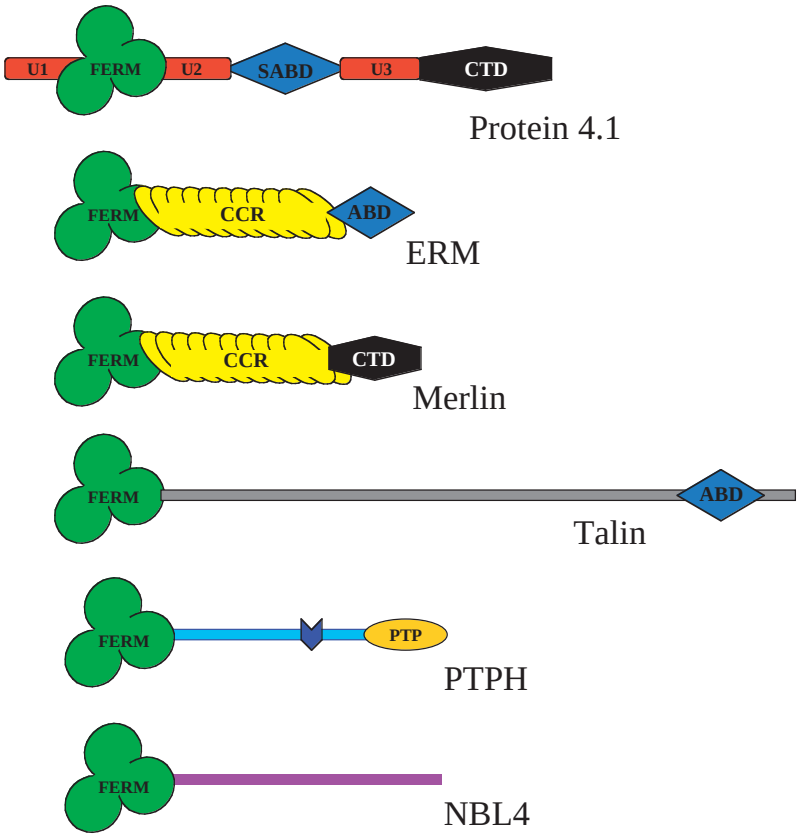

Fig. 1. Structural domains of selected members of the Protein 4.1 superfamily. The defining characteristic of all members is the highly homologous N-terminal FERM domain. The degree of similarity of FERM domains compared with that of the founding member of this superfamily, Protein 4.1R, are as follows: Protein 4.1N, 71%; Protein 4.1G, 74%; Protein 4.1B, 73%; ezrin/moesin/radixin, 24-32%; merlin, 28%; talin, 20%; PTPH, 37%; and NBL4, 40%. Merlin is structurally similar to the ERM proteins and these four proteins comprise the ERM subfamily. Domains shown in the prototypical Protein 4.1 are conserved among all members in the Protein 4.1 subfamily. Talin, PTPH, and NBL4 proteins are shown for comparison. ABD, actin-binding domain; CCR, predicted coiled-coil region; CTD, carboxyl terminal domain; FERM, Protein 4.1-ezrin-radixin-moesin domain; PTP, protein tyrosine phosphatase; SABD, spectrin-actin binding domain; U1, 2, 3, unique regions.

conserved domains, 4.1 proteins possess several unique domains: U1, U2 and U3. Although the functions of these domains are not known, their sequences are distinct from each other and thus might specify unique protein interactions that underlie the functional differences between Protein 4.1 family members.

One of the main functions of Protein 4.1 family members is the structural stabilization of the cell membrane, which has been extensively characterized in the erythrocyte. A decrease in 4.1R expression as a consequence of a chromosomal mutation results in hereditary elliptocytosis, a disorder characterized by pronounced hemolysis, splenomegaly and abnormally shaped red blood cells (Tchernia et al., 1981). This abnormal erythrocyte phenotype has also been documented in Protein 4.1R null mice (Shi et al., 1999). Consistent with a role in membrane stabilization, the FERM, SABD and CTD domains have been shown to mediate membrane-cytoskeleton interactions through interactions with integral membrane proteins. Protein 4.1 interacts with the Band 3 protein (Pasternack et al., 1985), glycophorin C and glycophorin D (Hemming et al., 1994; Marfatia et al., 1995), p55 (Marfatia et al., 1995; Pasternack et al., 1985), CD44 (Nunomura et al., 1997) and calmodulin (Tanaka et al., 1991) through N-terminal FERM domain sequences. Protein 4.1 binds spectrin and actin

Table 1. Protein 4.1/ERM family molecules

Protein	Molecular mass (kDa)	Chromosome	Adult tissue distribution	Subcellular localization
4.1B, type II brain 4.1, KIAA0987, EPB41L3 (DAL-1)	125, 145	18p11.3	Brain, heart, lung, kidney, intestine, testis, adrenal gland	Plasma membrane at regions of cell-cell contact
4.1R, EPB41	80, 135	1p36.2-p34	Erythrocytes, brain	Plasma membrane, nucleus, spindle poles of mitotic cells, centrosomes
4.1N, type I brain 4.1, KIAA0338, EPB41L1	100, 135	20q11.2-q12	Brain, peripheral nerve	Juxta-membrane localization, discrete foci along the neuronal dendrites, nuclear mitotic apparatus
4.1G, EPB41L2	95, 140	6q23	Heart, brain, placenta, lung, skeletal muscle, kidney, pancreas, gonads	Cytoplasmic, perinuclear, centrosome
Merlin, Schwannomin	69	22q12.2	Brain, lens, sciatic nerve, blood vessels, adrenal gland, Schwann cells, peripheral nerve, gonads	Perinuclear, plasma membrane, filopodia, regions of cell/substrate adhesion
Ezrin, Cytovillin	81	6q25-q26	Brain, kidney, intestine, lung, peripheral nerve, Schwann cells	Plasma membrane, apical microvilli, actin-containing surface structures, cell-cell adherens junctions
Radixin	82	11q23	Brain, kidney, liver, lung, thymus, peripheral nerve, Schwann cells gonads, skin	Plasma membrane, apical microvilli, actin-containing surface structures, cell-cell adherens junctions
Moesin	78	Xq11.2-q12	Brain, endothelial cells, heart, kidney, muscle, lung, liver, spleen, peripheral nerve, Schwann cells	Plasma membrane, apical microvilli, actin-containing surface structures, cell-cell adherens junctions

and potentiates interactions of spectrin tetramers with F-actin through its SABD (Ohanian et al., 1984), whereas Protein 4.1N does not interact with spectrin (Gimm et al., 2002). Protein 4.1 molecules also associate with tubulin through SABD sequences (Correas and Avila, 1988) and with FKBP13 (13 kDa FK506-binding protein) (Walensky et al., 1998) through CTD residues. As a result of these interactions, Protein 4.1 molecules play important structural and regulatory roles in the stabilization and assembly of the cell membrane. The association of Protein 4.1 with spectrin/actin and glycophorin C/p55 complexes appears to be essential for the maintenance of normal erythrocyte morphology, and the interaction with tubulin suggests that Protein 4.1 molecules may regulate microtubule architecture.

Protein 4.1 molecules also exhibit unique binding properties and differentially associate with a variety of proteins. Protein 4.1R interacts with the novel centrosomal protein CPAP (Hung et al., 2000), the eukaryotic translation initiation factor 3 (eIF3) complex (Hou et al., 2000), the zona occludens protein ZO-2 (Mattagajasingh et al., 1999) and the pIc1n protein involved in cellular volume regulation (Tang and Tang, 1998). In contrast, Protein 4.1N associates with the nuclear mitotic apparatus protein NuMA (Ye et al., 1999), regulates AMPA receptor GluR1 subunit surface expression (Shen et al., 2000) and binds to a nuclear phosphoinositide 3-kinase enhancer protein (Ye et al., 2000).

Members of the ERM family contain three main domains, including a FERM domain or N-terminal ERM association domain (N-ERMAD) and a C-terminal actin-binding domain (C-ERMAD), which are separated by a predicted coiled-coil (α-helical) domain (Fig. 1). Structural studies of the FERM domain of moesin revealed that it has three distinct subdomains: F1, F2 and F3 (Edwards and Keep, 2001). F1 shares structural features with ubiquitin, and F2 is homologous to acyl-CoA-binding proteins, whereas F3 shares sequence similarity with phosphotyrosine binding (PTB), pleckstrin homology (PH) and Enabled/VASP homology 1 (EVH1) domains.

ERM proteins can form intramolecular and intermolecular associations that are regulated by protein phosphorylation or lipid interactions. There are two intramolecular associations: one between the N-terminus and the C-terminus and the other within the N-terminus domain itself. The N-terminal ~300-residue FERM domain of ERM molecules can tightly associate with the C-terminal ~100 residues of other ERM proteins (Gary and Bretscher, 1995; Magendantz et al., 1995). In a hypophosphorylated state, ERM proteins adopt a ‘closed’ conformation that masks the binding sites for actin and CD44 (Hirao et al., 1996). Phosphorylation separates the N- and C-termini to result in an ‘open’ form, which permits interactions with the actin cytoskeleton and other proteins (Pearson et al., 2000). In this manner, phosphorylation of ezrin (Thr567), radixin (Thr564) or moesin (Thr558) by Rho kinase reduces the interaction between its N- and C-termini (Matsui et al., 1998). Furthermore, mutations in moesin that mimic Thr558 phosphorylation result in the formation of persistent microvillar structures (Oshira et al., 1998), suggesting that phosphorylation-dependent ‘unfolding’ of ERM proteins is important for their ability to modulate actin-cytoskeleton-associated processes. Lipid binding can also modulate these intramolecular associations. Binding of the FERM domain to phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] stimulates unfolding of ERM proteins and their subsequent association with adhesion proteins (Hamada et al., 2000).

ERM proteins also associate with several types of transporter molecule through PDZ domain sequences present in these transporters. EBP50 (ERM-binding phosphoprotein 50), also known as NHERF1 (Na^+/H^+ exchanger regulatory factor 1), is a homologue of a rabbit protein cofactor involved in renal brush-border Na^+/H^+ exchange. EBP50 colocalizes with actin and ERM proteins in actin-rich structures and can be immunoprecipitated with ERM proteins from placental microvilli. (Reczek et al., 1997; Murthy et al., 1998; Nguyen et al., 2001). Another PDZ-containing protein, E3KARP (exchanger 3 kinase A regulatory protein) or NHERF2, binds the N-terminus of ERM proteins (Yun et al., 1998; Voltz et al., 2001). The binding sites for EBP50 and E3KARP in full-length ezrin are masked by intramolecular N/C-ERMAD self-association. Growth factors stimulate the phosphorylation of a C-terminal residue in ezrin (and moesin), resulting in ezrin activation (Bretscher, 1999). Upon activation, ezrin unfolds and can then bind EBP50/E3KARP through N-terminal residues and F-actin through C-terminal residues (Reczek and Bretscher et al., 1998). NHERF proteins perform overlapping functions as regulators of transmembrane receptors, transporters and other proteins localized at or near the plasma membrane, where ERM proteins are enriched (Voltz et al., 2001). The relationship between NHERF binding and ERM function is not known.

Merlin

Neurofibromatosis 2 (NF2) is an autosomal dominant inherited cancer predisposition syndrome characterized by schwannomas of the eighth cranial nerve, as well as schwannomas of other cranial nerves, meningiomas and ependymomas (Evans et al., 2000), and affects approximately 1 in 35,000 individuals. The gene responsible for neurofibromatosis 2 encodes a 595-residue protein called merlin or schwannomin (Trofatter et al., 1993; Rouleau et al., 1993).

Analysis of the predicted amino acid sequence of merlin reveals three domains: a FERM domain (residues 1-302), an α-helical region (residues 303-478), and a unique C-terminal domain (residues 479-595). The FERM domain is believed to be responsible for membrane binding in a PtdIns(4,5)P_2-dependent manner (Hamada et al., 2000). Crystallographic analysis of the merlin FERM domain demonstrated similarities to that of ezrin and moesin (Fig. 2A). However, the merlin FERM domain also contains a seven residue Blue Box (BB) (residues 177-183, YQMTPEM), which is identical in human and *Drosophila* merlin, but not conserved in other ERM proteins (Shimizu et al., 2002). Analysis of the crystal structure of the merlin FERM domain demonstrated the existence of three well-defined subdomains: A, B and C. Asp70 in the A subdomain can form a salt bridge with Arg291 and Lys289 in the C subdomain. Other ERM proteins lack this aspartate residue at this position and are instead rich in aromatic residues, which effectively pushes the A subdomain loop away from the C subdomain. These differences in sequence and 3D structure might be responsible for merlin’s unique function as a tumor suppressor and its distinct protein-protein interactions (Kang et al., 2002).

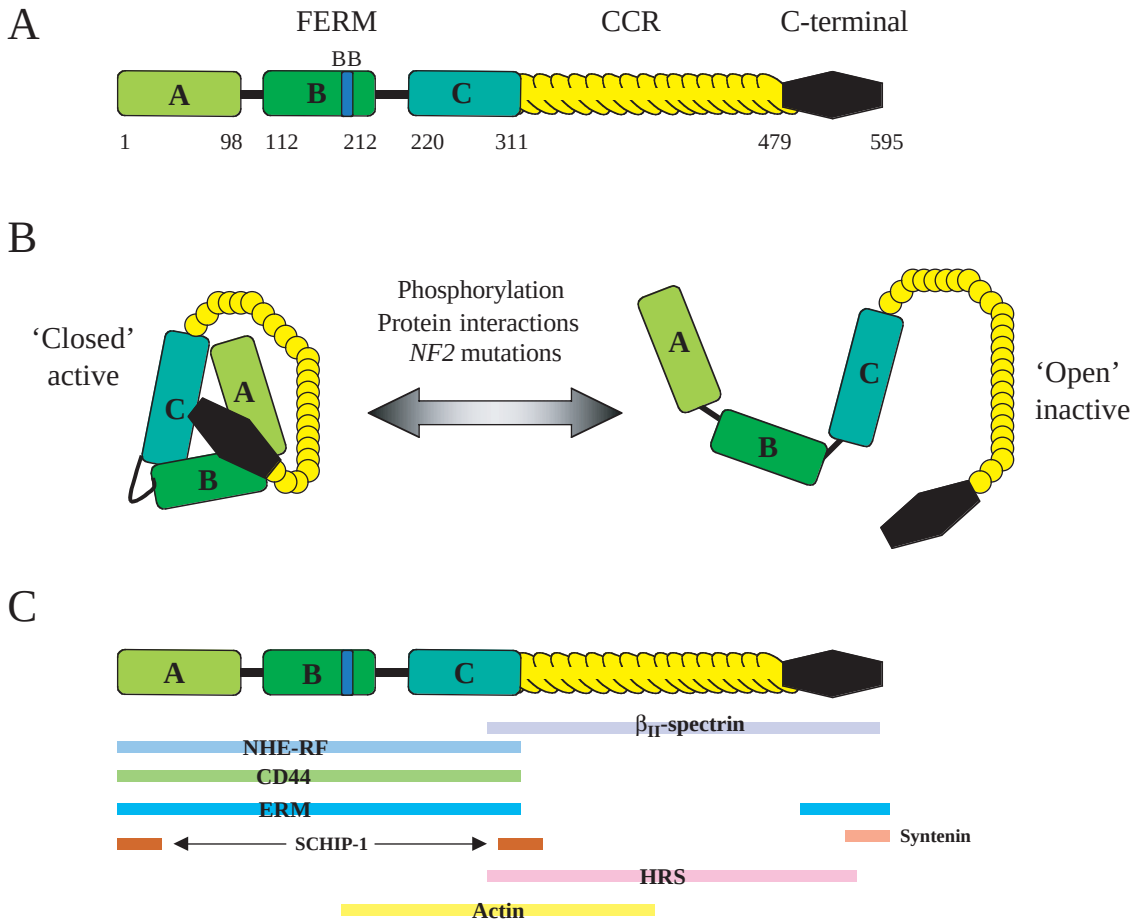

Fig. 2. Merlin structure and interactions. (A) Merlin contains three conserved protein-protein interaction domains: a FERM domain in its N-terminus and a C-terminal domain (CTD) separated by a coiled-coil (α-helical) region. Crystallography showed that the merlin FERM domain contains three subdomains, which exhibits a cloverleaf architecture. Merlin FERM has a unique 'Blue Box' (BB, residues 177-183) compared with other ERM proteins. (B) Merlin can adopt two conformations: a 'closed' active and 'open' inactive form. Merlin can switch from these two conformations as a result of phosphorylation, lipid binding, protein interactions or *NF2* mutations. (C) Merlin interacts with several molecules, including NHE-RF, βII-spectrin, CD44, other ERM proteins, SCHIP-1, HRS, actin and syntenin, which may affect merlin function as a growth suppressor. The proposed domains in merlin that mediate these interactions are depicted.

Two major isoforms of merlin result from alternative splicing of exon 16. Isoform 1 is a 595-residue protein encoded by exons 1-15 and 17. Isoform 2 contains exon 16, which inserts 11 unique C-terminal residues followed by a stop codon that prevents translation of exon 17, generating a 590-residue protein in which the first 579 residues are identical to isoform 1 (Bianchi et al., 1994). Other splicing variants have also been identified on the RNA level, but have not been detected by western blotting. It is not known whether these other isoforms are expressed under normal physiological conditions or whether they contribute to merlin function.

In order to function as a tumor suppressor, merlin must form two intramolecular associations. The first requires the binding of the N-terminus to the C-terminus, whereas the second involves folding within the N-terminal domain itself (Fig. 2B). The association of the N- and C-termini of merlin involves residues 302-308 within the C subdomain and exon 17 sequences, whereas residues within subdomains A and C participate in the intra-N-terminus interaction (Gutmann et al., 1999a; Sherman et al., 1997). Merlin isoform 2 is not capable of head-to-tail self-association (Sherman et al., 1997; Gonzalez-Agosti et al., 1999). Folding of the merlin N-terminus is required for the proper localization of the protein beneath the plasma membrane and influences the interaction between merlin and actin (Brault et al., 2001). However, the merlin N-/C-terminal domain self-association is relatively weak and dynamic, such that the C-terminus has a relatively higher affinity for the N-terminus of ezrin than for its own N-terminus (Nguyen et al., 2001). The heteromeric interactions between merlin and ezrin might regulate merlin function (Meng et al., 2000), perhaps by forming complexes that differentially modulate the ability of merlin to bind to critical effector or regulatory molecules.

Merlin in tumorigenesis and development

Unlike most Protein 4.1 family members, merlin functions as a tumor suppressor. *NF2* inactivation and loss of merlin expression are associated with the development of schwannoma and meningioma tumors (reviewed by Reed and Gutmann, 2001). Moreover, the *NF2* gene has also been implicated in the development of non-NF2-associated

meningiomas and schwannomas; merlin expression is lost in 60% of sporadic meningiomas and 80% of sporadic schwannomas (Bianchi et al., 1994; Ruttledge et al., 1994; Twist et al., 1994; Merel et al., 1995; Gutmann et al., 1997). In addition, re-expression of wild-type merlin in *NF2*-deficient schwannoma and meningioma cell lines in vitro results in growth suppression (Sherman et al., 1997; Ikeda et al., 1999), and mice with a targeted mutation in the *Nf2* gene ($Nf2^{+/-}$ mice) are prone to the development of malignant and metastatic tumors (McClatchey et al., 1998). This $Nf2^{+/-}$ tumor phenotype can be dramatically accelerated in the presence of a targeted mutation in another tumor suppressor gene, p53. In addition, tissue-specific inactivation of *Nf2* in mouse Schwann cells or leptomeningeal cells is sufficient for the development of schwannomas (Giovannini et al., 2000) and meningiomas (Kalamarides et al., 2002), respectively.

In addition to functioning as a negative growth regulator in tumor formation, the *NF2* gene plays an important role in embryogenesis and tissue differentiation. Complete inactivation of the mouse *Nf2* gene results in embryonic lethality between day 6.5 and day 7.0 (McClatchey et al., 1997). These mice exhibit a collapsed extraembryonic region and the absence of organized extraembryonic ectoderm. Similarly, *Drosophila* merlin is required in posterior follicle cells to initiate axis formation. Defects in nuclear migration and mRNA localization in the oocyte are found in *Drosophila* merlin mutants (MacDougall et al., 2001). In *Drosophila*, merlin may additionally regulate cell proliferation through interactions with another member of the Protein 4.1 family, *expanded* (McCartney et al., 2000).

Merlin interacts with the actin cytoskeleton

In contrast to other ERM members, merlin lacks a conventional actin-binding site at its C-terminus; in common with ERM proteins, however, it has alternative actin-binding sites within its FERM domain (Brault et al., 2001; Xu and Gutmann, 1998) and these can mediate direct interactions with the actin cytoskeleton. Although merlin exhibits weak direct actin binding compared with other ERM proteins, it may indirectly associate with the actin cytoskeleton by interacting with βII-spectrin, a known F-actin-binding protein (Scoles et al., 1998).

In support of an actin cytoskeleton function for merlin, *NF2*-deficient schwannoma cells exhibit dramatic alterations in actin cytoskeleton organization (Pelton et al., 1998). In these cells, the re-introduction of wild-type, but not mutant, merlin reverses these abnormalities (Bashour et al., 2002). In addition, regulated overexpression of wild type, but not mutant, merlin in rat schwannoma cells results in transient alterations in F-actin organization during cell spreading, abnormalities in cell attachment and reduced cell motility (Gutmann et al., 1999b; Gutmann et al., 2001a).

Merlin protein interactions

In an effort to determine how merlin functions as a tumor suppressor, several groups have identified potential merlin-interacting proteins, including SCHIP-1 (Schwannomin-interacting protein 1), NHERF (Na^+-H^+ exchanger regulatory factor), βII-spectrin (also known as fodrin), CD44, syntenin, paxillin and HRS (hepatocyte growth factor-regulated tyrosine kinase substrate; also known as HGS) (Goutebroze et al., 2000; Fernandez-Valle et al., 2002; Jannatipour et al., 2001; Murthy et al., 1998; Scoles et al., 2000). The regions of merlin important for most of these associations are illustrated in Fig. 2C. Of these potential interactors, four deserve special mention.

Paxillin binds to merlin residues 50-70 contained within exon 2 and facilitates the localization of merlin to the cell membrane where it can interact with cell surface proteins, such as CD44 and β1-integrin (Fernandez-Valle et al., 2002; Obremski et al., 1998). Syntenin is an adaptor protein that couples transmembrane proteoglycans to cytoskeletal components. Syntenin specifically interacts with merlin isoform 1 (Jannatipour et al., 2001), which suggests a possible link between 'active' merlin and membrane protein signaling through the actin cytoskeleton. HRS interacts with merlin both in vitro and in vivo (Scoles et al., 2000). HRS is a 115 kDa tyrosine phosphorylated protein localized to the cytoplasmic surface of early endosomes and is probably involved in the regulation of endocytosis and exocytosis (Clague and Urbe, 2001; Hayakawa and Kitamura, 2000; Raiborg et al., 2001; Urbe et al., 2000). In addition, HRS has been suggested to function in the TGFβ and EGFR (epidermal growth factor receptor) signaling pathways (Chin et al., 2001; Miura et al., 2000). It is hypothesized that HRS mediates downregulation of EGFR at the cell membrane by increasing EGFR internalization through an interaction with recruiting sorting nexin 1 (SNX1) (Clague and Urbe, 2001). Regulated overexpression of HRS in rat schwannoma cells has the same consequences as merlin overexpression (Gutmann et al., 2001b), raising the possibility that HRS participates in merlin growth suppression.

CD44 is a polymorphic transmembrane glycoprotein that functions as a receptor for hyaluronic acid (Peach et al., 1993). It is involved in cell adhesion and trafficking as well as in tumor motility and progression. Merlin interacts in vitro and in vivo with the cytoplasmic tail of CD44 (Sainio et al., 1997). CD44 also interacts with several guanine-nucleotide-exchange factors (GEFs) for Rho family GTPases, such as Tiam-1 (Bourguignon et al., 2000) and Vav2 (Bourguignon et al., 2001). The interaction of CD44 with these GEFs leads to activation of Rac1 and, under certain conditions, results in increased Rho activation and altered ERM protein-plasma membrane associations (Hirao et al., 1996). In this fashion, increased Rho activity could result in phosphorylation of the C-terminus of ERM proteins to regulate their head-to-tail associations and function (Matsui et al., 1998).

Protein 4.1B/DAL-1

DAL-1 (differentially expressed in adenocarcinoma of the lung) was originally identified by differential display RT-PCR as a 750 bp gene fragment whose expression was absent in several non-small cell lung carcinomas when compared with matched normal tissue (Tran et al., 1999). The reintroduction of DAL-1 into non-expressing non-small cell lung carcinoma cell lines resulted in a suppression of growth (Tran et al., 1999), suggesting that DAL-1 functions as a negative growth regulator. Subsequent analysis revealed that DAL-1 represents a fragment of the Protein 4.1B gene found on chromosome 18p11.3 (Gutmann et al., 2001c). The alignment of DAL-1

with Protein 4.1B demonstrates that Protein 4.1B contains unique sequences not found in DAL-1 (Tran et al., 1999) (Fig. 3). DAL-1 is represented by residues Met^{110} (the initiating methionine residue) through Ser542; it has a deletion of the N- and C-terminal domains as well as internal deletions within the U2 and SAB domains. Despite these deletions, DAL-1 contains all the residues necessary for the tumor suppressor properties of Protein 4.1B. In common with other Protein 4.1 molecules, Protein 4.1B contains an N-terminal FERM domain and a SABD. Three interspersed unique regions (U1, U2 and U3) are also found. Using specific antibodies, Protein 4.1B is a 125-145 kDa molecule localized to the plasma membrane near points of cell-cell contact (Tran et al., 1999) suggesting a role in cell-membrane-mediated processes. The fact that overexpression of DAL-1 impairs cell motility and disrupts the actin cytoskeleton during cell spreading is consistent with this idea (Gutmann et al., 2001c).

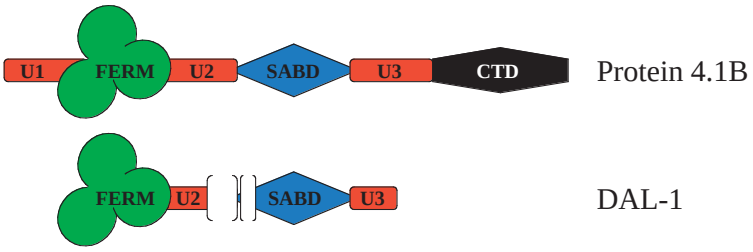

Fig. 3. Alignment of Protein 4.1B and DAL-1. Protein 4.1B contains unique sequences not present in DAL-1. DAL-1 is represented by residues M^{110} and S^{542}, with deletions in the N- and C-termini and internal deletions within the U2 and SAB domains. Brackets denote internal sequences absent in DAL-1.

The tumor suppressor function of DAL-1/Protein 4.1B has recently been documented. Loss of heterozygosity has been found in the chromosome 18p11.3 region where DAL-1 maps in 38% of lung, brain and breast tumors (Tran et al., 1999). In addition, the reintroduction of DAL-1/Protein 4.1B into DAL-1-deficient lung cancer (Tran et al., 1999) or meningioma cell lines reduces cell proliferation (Gutmann et al., 2001c). Protein 4.1B loss of heterozygosity (LOH) is a common genetic alteration in meningiomas, regardless of histological grade, indicating that Protein 4.1B inactivation might be an early event in meningioma tumorigenesis (Perry et al., 2000; Gutmann et al., 2000). However, the ability of Protein 4.1B/DAL-1 to function as a negative growth regulator is tissue-specific. Although merlin is able to suppress cell proliferation in schwannoma cells, overexpression of DAL-1 has no effect (Gutmann et al., 2001c). This is consistent with the observation that, in contrast to merlin, Protein 4.1B expression is not lost in sporadic schwannomas (Gutmann et al., 2000).

Although merlin has been shown to interact with several proteins integral to cell signaling, less is known about Protein 4.1B. To provide insight into the function of Protein 4.1B/DAL-1, efforts have been made to characterize potential interacting proteins that might mediate the Protein 4.1B growth inhibitory signal. Because of the high homology between Protein 4.1 family members, previously described merlin-binding partners have been assayed for their ability to interact with DAL-1, the fragment of Protein 4.1B known to contain the minimal growth suppression domain. In common with merlin, Protein 4.1B/DAL-1 has been shown to interact in vitro with ezrin, radixin and moesin, as well as with the N-terminus of merlin, possibly reflecting the ability of all members of the Protein 4.1B family to form intra- and intermolecular complexes (Gutmann et al., 2001c). Moreover, Protein 4.1B/DAL-1 also interacts with CD44 (V.A.R. and D.H.G., unpublished) as has been previously reported for Protein 4.1R (Numomura et al., 1997) as well as βII-spectrin (Gutmann et al., 2001c), which indicates that Protein 4.1B and merlin might signal in a similar manner. However, Protein 4.1B/DAL-1 does not interact with several known merlin-interacting proteins, including SCHIP-1 (Gutmann et al., 2001c).

The demonstration that DAL-1/Protein 4.1B interacts with a subset of merlin-binding proteins and functions in a cell-type-specific manner raises the possibility that DAL-1/Protein 4.1B associates with unique proteins that are specific to its function as a negative growth regulator. Recently, DAL-1 was found to interact with 14-3-3 molecules both in vivo and in vitro (Yu and Robb et al., 2002), which are involved in cell cycle regulation (reviewed by Muslin and Xing, 2000). Moreover, this interaction was specific to DAL-1/Protein 4.1B and was not observed with other Protein 4.1 family members (Yu and Robb, 2002). Since CD44 and 14-3-3 have each been implicated in mitogenic signaling pathways, the association of Protein 4.1B with these proteins might be important for transduction of the Protein 4.1B growth inhibitory signal.

Other Protein 4.1 growth suppressors

The fact that at least two members from two different branches of the Protein 4.1 superfamily are tumor suppressor genes raises the intriguing possibility that other members of the Protein 4.1 superfamily also play an important role in cell growth regulation. Recent observations have lent credence to this idea. Recent research has demonstrated that the Protein 4.1R gene is mutated, silenced, or has its intronic sequence changed in 14 of 72 neuroblastomas studied (Huang et al., 2001). The finding that different Protein 4.1 members are mutated or silenced in a variety of diverse tumor types suggests that Protein 4.1 molecules might be differentially involved in pathogenesis of multiple distinct cancers.

Proposed model of Protein 4.1 molecule growth suppression

Although the precise signal transduction pathways that mediate Protein 4.1 growth suppression have not been fully defined, a clearer picture of the function of merlin as growth regulator is emerging. We propose a model in which CD44 links growth signals from plasma membrane to the nucleus by interacting with ERM proteins and merlin (Morrison et al., 2001; Sherman and Gutmann, 2001). This model envisions that specific cellular and environmental conditions are required for effective merlin growth regulation (Fig. 4).

In this model, growth permissive conditions enable the activation of small GTPase molecules, such as Rac and Rho, which results in phosphorylation of ERM proteins, perhaps on specific C-terminal threonine or serine residues. Phosphorylated and ‘unfolded’ ERM proteins bind to CD44 in

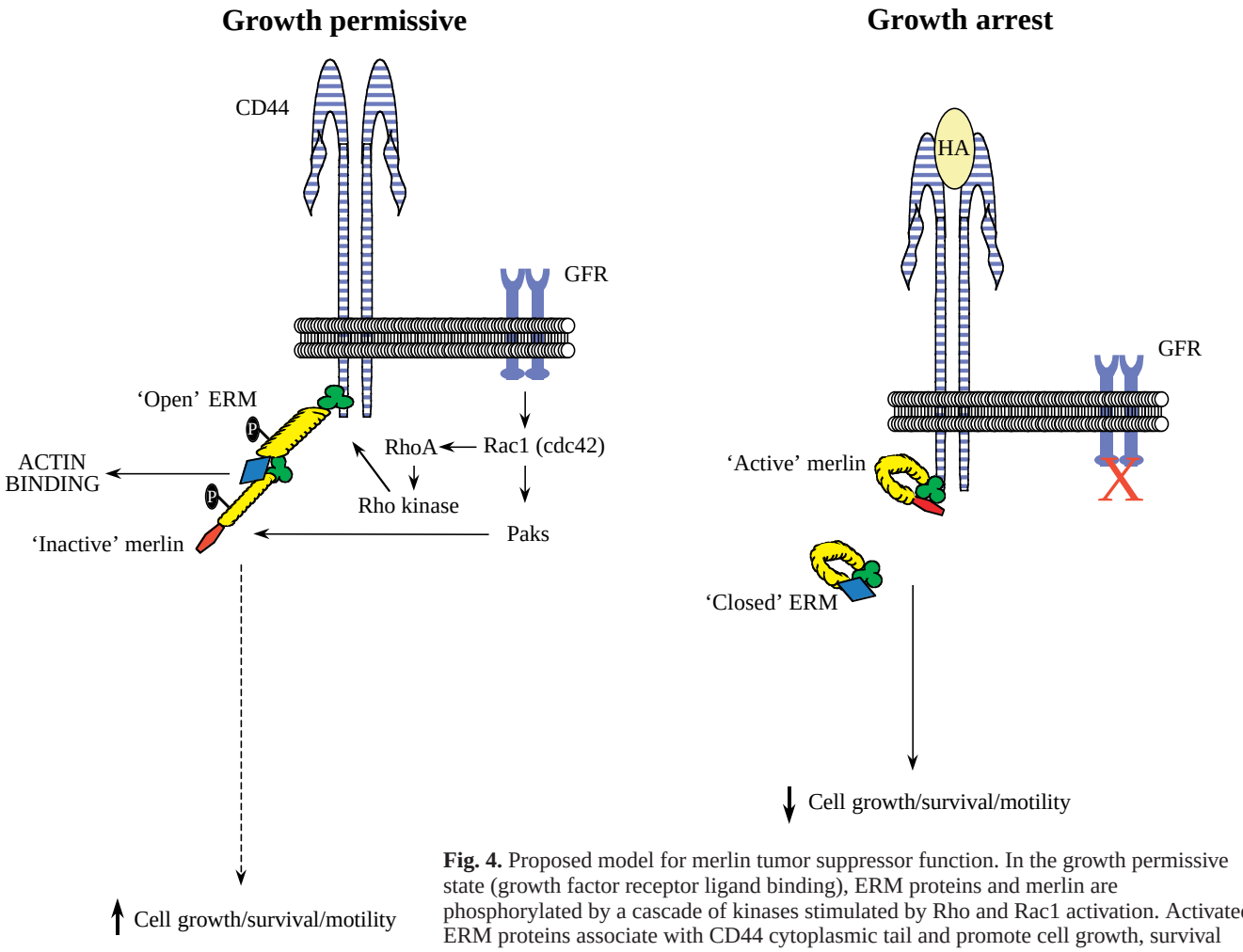

Fig. 4. Proposed model for merlin tumor suppressor function. In the growth permissive state (growth factor receptor ligand binding), ERM proteins and merlin are phosphorylated by a cascade of kinases stimulated by Rho and Rac1 activation. Activated ERM proteins associate with CD44 cytoplasmic tail and promote cell growth, survival and motility. 'Open' phosphorylated merlin resides in an inactive conformation and may associate with ERM proteins. In the growth arrest state (high molecular weight hyaluronic acid binding to CD44 receptor), this protein kinase cascade is not activated and ERM proteins are not phosphorylated. Merlin, in the hypophosphorylated form, is 'closed' and active. 'Active' merlin interacts with CD44 to facilitate inhibition of cell growth and motility. ERM proteins in the 'closed' conformation would be predicted to dissociate from the CD44 complex. GFR, growth factor receptor; HA, hyaluronic acid.

this activated conformation, leading to cellular remodeling and facilitating cell proliferation (Matsui et al., 1998; Matsui et al., 1999). Under these growth permissive conditions, merlin is also phosphorylated, perhaps by Rac1-dependent Pak activation, resulting in an 'open' and 'inactive' merlin molecule incapable of negatively regulating cell growth, but still able to bind ERM proteins (Morrison et al., 2001; Shaw et al., 2001; Kissil et al., 2002; Xiao et al., 2002). As ERM proteins associate with CD44 at low cell density and the merlin C-terminus has a high affinity for the N-terminus of ezrin (Nguyen et al., 2001), this 'open' conformation of ezrin might serve to retain merlin in an 'inactive' state at the plasma membrane under growth-permissive conditions.

In contrast, when cells are stimulated to undergo growth arrest by cell contact or specific extracellular matrix cues (e.g. high-molecular-weight hyaluronic acid), merlin and perhaps ERM proteins both exist in hypophosphorylated states, resulting in molecules in the 'closed' conformation (Morrison et al., 2001). This would favor binding of merlin to the cytoplasmic tail of CD44 to promote cell growth suppression (Sherman and Gutmann, 2001). In this growth inhibitory state, ERM proteins (ezrin) might not be as tightly associated with CD44 (Morrison et al., 2001). This model predicts that the phosphorylation state of merlin is modulated by growth arrest signals, such as confluency and serum deprivation, with the hypophosphorylated form being associated with growth arrest (Shaw et al., 1998).

On account of the striking sequence similarity among Protein 4.1 family members, it is conceivable that the productive interaction of select Protein 4.1 members (e.g. Protein 4.1B) and transmembrane molecules (e.g. CD44) also transduces their growth inhibitory signals. Based on available data, it is premature to propose a model of growth suppression for all Protein 4.1 growth suppressors. Further study of the complex relationship between growth regulatory members of the Protein 4.1 family and their binding partners will undoubtedly yield important insights into the mechanisms that underlie context-dependent growth arrest. With an improved understanding of the critical protein interactions important for Protein 4.1 molecule growth suppression and the processes that

modulate Protein 4.1 molecule activity, it is conceivable that novel and targeted cancer therapies may be developed.

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