

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

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Abstract

The band 4.1 domain was first identified in the red blood cell protein band 4.1, and subsequently in ezrin, radixin, and moesin (ERM proteins) and other proteins, including tumor suppressor merlin/schwannomin, talin, unconventional myosins VIIa and X, and protein tyrosine phosphatases. Recently, the presence of a structurally related domain has been demonstrated in the N-terminal region of two groups of tyrosine kinases: the focal adhesion kinases (FAK) and the Janus kinases (JAK). Additional proteins containing the 4.1/JEF (JAK, ERM, FAK) domain include plant kinesin-like calmodulin-binding proteins (KCBP) and a number of uncharacterized open reading frames identified by systematic DNA sequencing. Phylogenetic analysis of amino acid sequences suggests that band 4.1/JEF domains can be grouped in several families that have probably diverged early during evolution. Hydrophobic cluster analysis indicates that the band 4.1/JEF domains might consist of a duplicated module of

~140 residues and a central hinge region. A conserved property of the domain is its capacity to bind to the membrane-proximal region of the C-terminal cytoplasmic tail of proteins with a single transmembrane segment. Many proteins with band 4.1/JEF domains undergo regulated intra- or intermolecular homotypic interactions. Additional properties common to band 4.1/JEF domains of several proteins are binding of phosphoinositides and regulation by GTPases of the Rho family. Many proteins with band 4.1/JEF domains are associated with the actin-based cytoskeleton and are enriched at points of contact with other cells or the extracellular matrix, from which they can exert control over cell growth. Thus, proteins with band 4.1/JEF domain are at the crossroads between cytoskeletal organization and signal transduction in multicellular organisms. Their importance is underlined by the variety of diseases that can result from their mutations.

Introduction

Attachment of proteins to membranes is necessary for the organization of cell structure, providing each cell with its characteristic shape and its capacity for movement. Work during the last decade has demonstrated that protein targeting to the membrane is also critical for signal transduction (1). The activation of a number of signaling pathways begins with the highly regu-

lated aggregation of specific proteins at the level of the membrane, allowing the initiation of cascades of enzymatic reactions. Thus, the study of the mechanisms responsible for membrane targeting of cytoplasmic proteins has become a fertile area of research. Many proteins have covalently attached fatty acids including palmitate, myristate, and isoprenoid derivatives, which can themselves be inserted in the lipidic bilayer (2). In some cases these interactions are regulated and proteins can cycle from a soluble to a membrane-bound state. Specific protein domains can also interact noncovalently with membrane phospholipids using pleckstrin homology (PH)

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domains, which bind phosphatidylserine polyphosphates (3), or C2 domains, which bind phosphatidylserine in the presence of Ca^{2+} (4). Membrane targeting of cytoplasmic proteins is also achieved by interaction of specific domains, including PSD-95/Dlg/Zo-1 (PDZ), WH1/EVH1, and death domains, with transmembrane proteins (5,6). SH2 and phosphotyrosine binding (PTB) domains play an important role by interacting with membrane-associated proteins when they are phosphorylated on tyrosine (7). Interestingly, the roles of lipids and proteins in membrane targeting are not separated, since phospholipids can regulate protein-protein interactions directly or indirectly (8). Moreover, some domains can interact with both lipids and proteins. One example is the PH domains that interact with phosphoinositides and with β/γ subunits of heterotrimeric G proteins (3,9).

The band 4.1 domain is a large (>300 residues) and relatively less characterized domain, capable of interaction with several types of proteins including transmembrane proteins, and in some cases with lipids (10,11). This domain was originally identified as the N-terminal chymotrypsin-resistant region of erythrocyte protein band 4.1, which is capable of binding to glycophorin C (12,13). It was then recognized in three related proteins, ezrin, radixin, and moesin (ERM), which also act as linkers between the actin cytoskeleton and the plasma membrane (10). The band 4.1 domain is present in many proteins (14,15) (Fig. 1). The acronym FERM (*Four-point-one/Ezrin/Radixin/Moesin*) has been suggested to refer to this domain (14). However, recent work has extended considerably the significance of the band 4.1 domain, showing that, despite a low level of sequence identity, significant structural relationships exist between the N-terminal region of focal adhesion kinases (FAK) and Janus kinases (JAK) (15). A relationship between the band 4.1 domain and FAK N-terminal region was also reported independently by means of a different approach (16). In this review, we will use the term band 4.1/JEF (JAK-ERM-FAK) to refer to this domain. We shall summarize the approaches that allowed identification of the relationship between the distant members of the band 4.1/JEF superfamily and the structural characteristics of this domain. Some features of the various proteins containing band 4.1/JEF domains and of their known partners are reviewed, and their common properties outlined. Finally, we shall indicate the impor-

tance of the mutations of these domains in human pathology.

Sequence and Structural Features of the Band 4.1/JEF Domain Superfamily

The band 4.1 domain has been defined on the basis of sequence similarities easily detected by automatic procedures such as BLAST or FASTA. This allowed the identification of its presence in ERM and proteins such as protein tyrosine phosphatases, talin, and unconventional myosins (10,14). However, the recognition of this domain in the N termini of FAK and JAK (15) was not possible by this approach and required the use of a bidimensional method of sequence analysis called hydrophobic cluster analysis (HCA) (17,18). The sensitivity of HCA within and below the twilight zone (i.e., ~25–30% sequence identity) originates from its ability to combine sequence comparison with secondary structure prediction. Indeed, the clusters formed by hydrophobic amino acids on the HCA bidimensional plot (Fig. 2) have been shown to correspond mainly to the internal faces of regular secondary structures in globular proteins (19). They constitute two-dimensional (2D) signatures that are much more stable, and therefore recognizable through human expertise, than signatures described from 1D sequence only (20). Therefore, at low levels of sequence identity, the method is particularly efficient in detecting similar folds by sorting out true relationships from background noise (18).

The starting point of the analysis was the finding of a close relationship between the N-terminal sequence of FAK and an uncharacterized human gene product termed KIAA0316 (15). This finding was decisive since, although clearly belonging to the FAK group (Fig. 3), the KIAA0316 sequence is a key linker between the FAK group and the band 4.1 domain of talin. In this respect, talin and related sequences form a distinct group intermediate between FAK and classical ERM band 4.1 domains (Fig. 3). On the other hand, the FAK group was also found to be related to the N-terminal sequence of JAKs. This latter group is distinguished by the long sequences that separate conserved blocks in the alignment of band 4.1/JEF domains (Fig. 2a) (15). Such intervening sequences of variable length interfere with the ability of current 1D methods to establish relationships between pro-

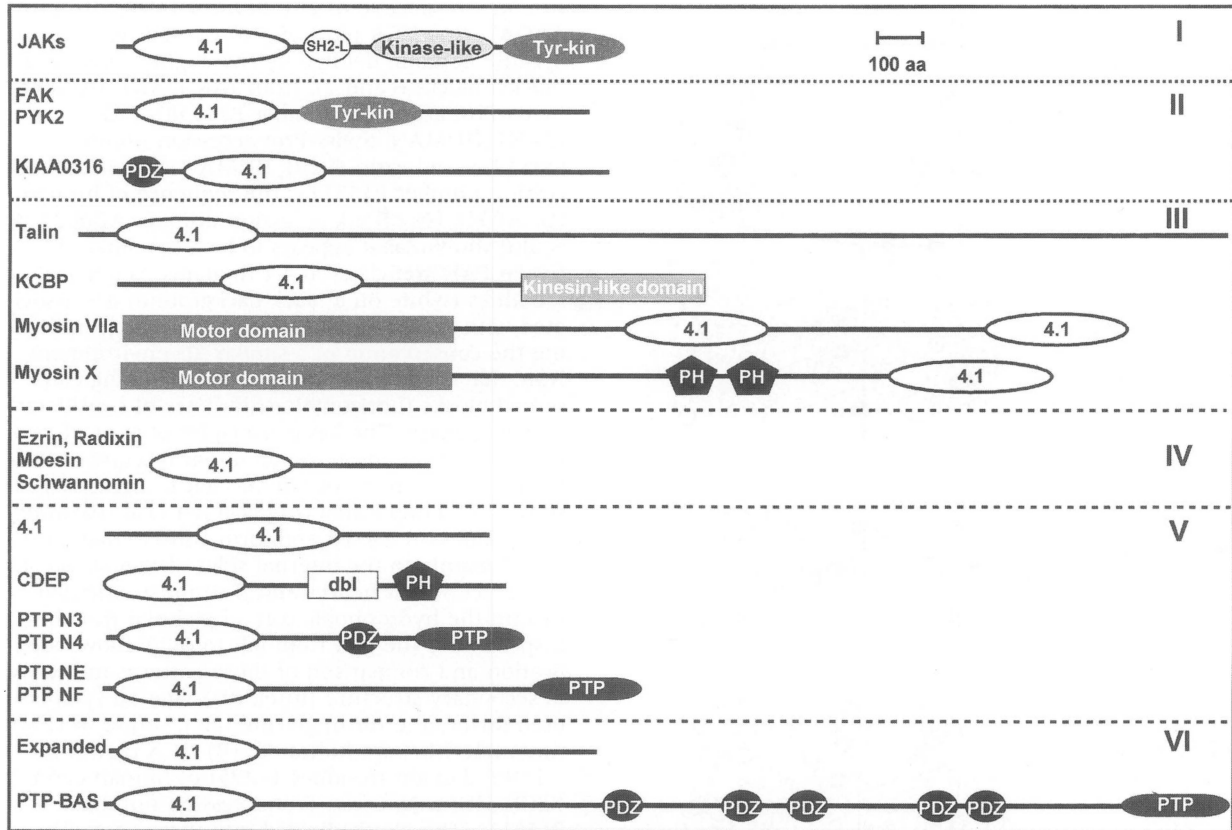


Fig. 1. Schematic domain organization of proteins with band 4.1 JEF domains. Examples of proteins with distinct domain organization are shown in six groups based on the sequence similarities of their band 4.1/JEF domain. Group I: JAK kinases; group II: FAK, PYK2, and KIAA0316, a non-characterized open reading frame; group III: talin, unconventional myosins, and plant kinesin-like calmodulin binding proteins (KCBP); group IV: the ERM proteins (ezrin, radixin, moesin) and the tumor suppressor merlin/schwannomin; group V: band 4.1, CDEP, and several protein tyrosine phosphatases (PTP); group VI: Expanded, a *Drosophila* protein, and

PTP-BAS, which have no close relationship with each other or with other proteins in the sequence of their band 4.1/JEF domain. Additional domains include the tyrosine kinase domain (Tyr-kin), the kinase-like domain (kinase-like), a domain structurally related to the Src-homology 2 domain (SH2-L), the PSD95-Dlg-ZO1-homology domain (PDZ), the pleckstrin homology domain (PH), and the protein tyrosine phosphatase domain (PTP). Other characterized regions, including those involved in coiled-coil interactions or in actin binding are not indicated because their sequence and limits are less well defined than those of globular domains.

teins because of gap penalties that these methods introduce, as they cannot, at least in noniterative procedures, discriminate between secondary structures and loop regions. The presence of a band 4.1-related domain at the N terminus of FAK was also detected independently by a different approach, using automatic comparison of query sequences to multiple alignments of known domains (16). However, this method could not detect the relationship of band 4.1 with JAK, presumably because of the variable length of sequences between conserved clusters.

As a consequence of this variability, the size of the band 4.1/JEF domain itself is variable,

ranging from ~300 residues in ERM proteins to ~400 residues in some JAKs. This large size, as well as the multiplicity of its potential partners, suggests that the band 4.1/JEF domain could be made up of several modules. A close examination of the HCA plots supports this hypothesis, showing that the band 4.1/JEF domain could consist of a duplicated module (Fig. 2b; see below). The low sequence identity shared by the two related subdomains indicates that this duplication is an ancient evolutionary event that occurred before the functional specialization of the various band 4.1/JEF domains. Moreover, in screening databases we were unable to find any

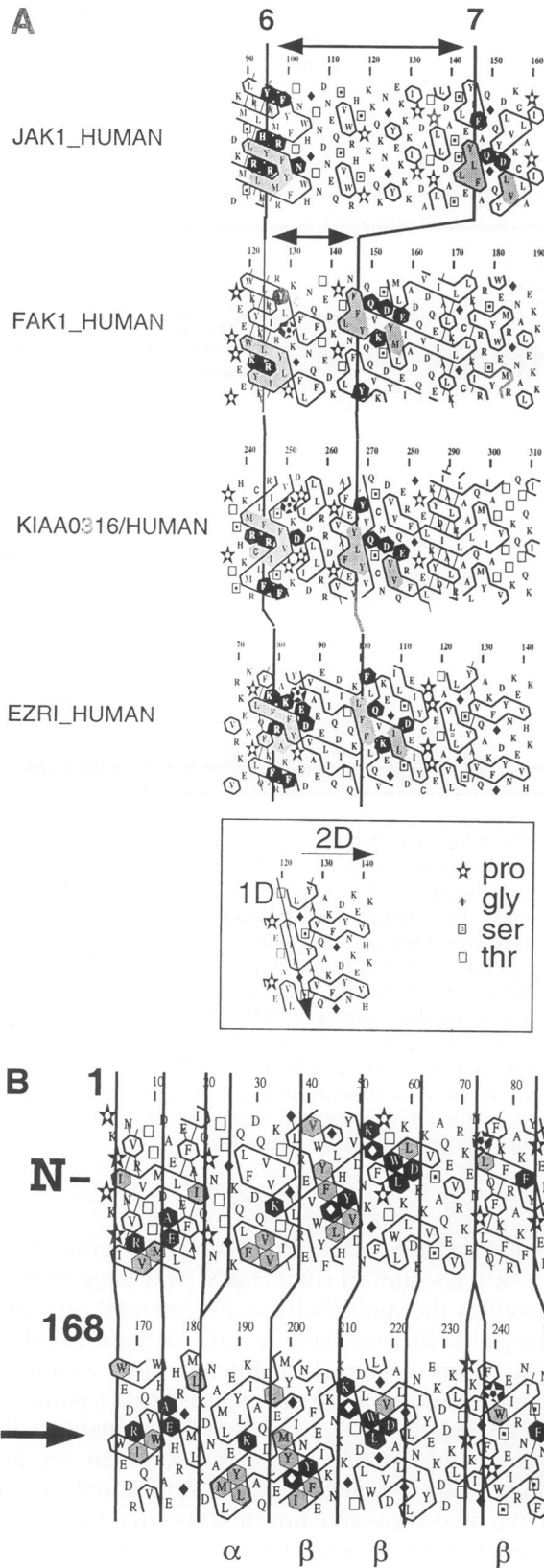


Fig. 2. Structure of the band 4.1/JEF domain (HCA). (A) HCA plots of a conserved region of the 4.1/JEF domain, defined by two highly conserved blocks (blocks 6 and 7), from JAK (JAK1_HUMAN, Swiss-Prot accession number P23458), FAK (FAK1_HUMAN, Swiss-Prot accession number Q05397), and ezrin (EZRI_HUMAN, Swiss-Prot accession number P15311). The sequence of human KIAA0316 (GenBank accession number AB002314) is also shown, as it appears to be a key linker between FAK and classical 4.1 domains. Sequence identities (white on a black background) are associated with cluster similarities (shaded gray), indicating the conservation of a similar 2D environment. Note that the intervening sequences linking conserved blocks (horizontal arrows) are of considerable variable length. The basic principles of HCA plots are indicated in the inset: the amino acid sequence (obliquely from top to bottom and left to right, 1D) is shown on a duplicated α -helical net (18). Hydrophobic residues are boxed and form clusters that correspond mainly to the internal sides of regular secondary structures (α helices and β strands), oriented toward the hydrophobic core of globular proteins. Inspection of the plot from left to right allows identification and comparison of those patterns indicative of secondary structure (inset, 2D). Special symbols used for proline (pro), glycine (gly), serine (ser) and threonine (thr) are indicated. (B) HCA plot of the 4.1/JEF domain (residues 1–295) of human ezrin (EZRI_HUMAN, Swiss-Prot accession number P15311). The plot is divided into three parts. The central hinge containing Tyr-145 (Y*), a substrate for tyrosine kinases, is set aside to highlight the similarities between the two aligned subdomains (residues 1–132 and 168–295). Secondary structure predictions are shown below the duplicated subdomains. Sequence identities (white on a black background) are associated with cluster similarities (shaded gray), indicating the conservation of a similar 2D environment. Special symbols used for proline, glycine, serine, and threonine are as indicated in panel A.

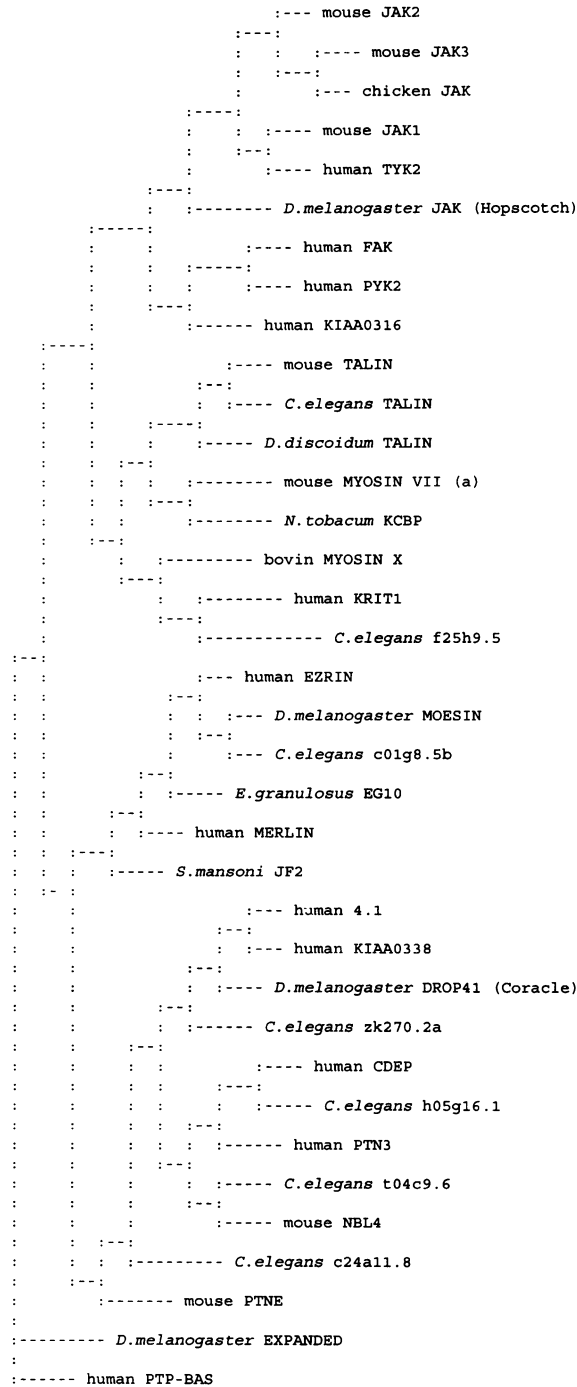


Fig. 3. Hypothetical phylogenetic tree of the band 4.1/JEF domains. Phylogenetic tree based on amino acid sequences aligned following (15) was constructed using the program PROTML in the MOLPHY package (137). Note that the longest intervening sequences (corresponding to loops between conserved blocks) were omitted from the alignment.

occurrence of one of the two subdomains independently, which supports the hypothesis of their ancient origin and suggests a structural in-

terdependence between them. Duplications have been described in a number of proteins. They are sometimes easily detected because of a high sequence identity between the subdomains, whereas in other cases they become apparent only after the experimental determination of the three-dimensional structure.

The band 4.1/JEF domains appear to consist of two structurally similar subdomains of ~130 residues linked by a short hinge region (block 9) of 30–35 residues, containing an invariant proline residue. The evidence for duplicated subdomains is visible on the HCA plots of human ezrin (Fig. 2b). Sequence conservation (e.g., ~13% sequence identity in the case of ERM) and hydrophobic cluster conservation are observed between the two putative subdomains. The hypothesis of a duplication is further supported by similar secondary structure predictions. Comparison of the sequence of the two putative subdomains highlights short conserved sequence motifs that are predicted to be related to the core structure of the domain or a key conserved function. These motifs, including the stretches /x//g/n, w/x/d and ///q/+ (where / stands for an hydrophobic residue; +, a positively charged residue; g, a glycine residue; q, a glutamine residue; w, a tryptophan residue; and x, a variable position) are all associated with predicted buried β strands. The evidence for an internal duplication is clear within the 4.1/ERM group and in the case of KIAA0316 (data not shown). However, it is much less conspicuous in the FAK and JAK groups, suggesting that the two subdomains have undergone a divergent evolution in these tyrosine kinases.

Proteins with Band 4.1/JEF Domains and Their Partners

Band 4.1 and Related Proteins

Band 4.1 contains a C-terminal actin/spectrin binding-domain and thus acts as a cross-linker between the cortical cytoskeleton and the membrane of erythrocytes, playing a critical role in the characteristic shape of these cells (12). The membrane partners of band 4.1 have been extensively studied. The main anchor site is the short intracellular C-terminal tail of glycoprotein C, a small transmembrane protein with a heavily glycosylated extracellular domain. The band 4.1 binding site of glycoprotein C has been mapped precisely (21,22) (Fig. 4a). The N-terminal domain of erythrocyte band 4.1 binds also to the

anion exchanger of erythrocyte membrane (band 3) (23) and to CD44, first identified as an anchoring site for ERM proteins (see below) (24). The interaction may involve a stretch of arginine residues in band 3 and acidic residues (LEEDY) in band 4.1 (25) (Fig. 4b). Another important partner of the band 4.1/JEF domain in red blood cells is the protein p55, which contains a PDZ, a SH3, and a guanylate-kinase domain and belongs to the MAGUK (membrane associated-guanylate kinases) family of proteins (21). p55 is capable of binding simultaneously the band 4.1/JEF domain by a short region between its SH3 and guanylate kinase domains (Fig. 4b) and the C terminus of glycophorin C by its PDZ domain (26,27) (Fig. 5a). Thus, a ternary complex between glycophorin C, band 4.1, and p55 may be a basic structural component allowing anchorage of the cortical cytoskeleton to the erythrocyte membrane (Fig. 5a). The sequence in p55 responsible for band 4.1 binding is conserved in *Drosophila* disc-large protein (Dlg) and its human homologue (H-dlg), which suggests that such interactions have been conserved during evolution (21). The formation of membrane-associated complexes containing band 4.1 is probably highly regulated, since the binding of band 4.1 to glycophorin C is increased by phosphatidylinositol 4,5-bisphosphate (PIP2) (13), whereas Ca^{2+} /calmodulin reduces its affinity for CD44 (24).

The role of band 4.1 is not restricted to erythrocytes. In fact, a number of different 4.1 proteins with distinct patterns of tissue expression and different intracellular localizations are generated from the same gene, as a result of two different sites of initiation of transcription and multiple alternatively spliced exons (28,29). In addition, several other genes code for related proteins. One of them is an ubiquitously expressed 4.1-G (general protein 4.1) which was identified as a protein interacting with FKBP-13 of the immunophilin family (30). Other as-yet uncharacterized gene products with high sequence similarity to band 4.1 include NBL4 (Novel Band 4.1-Like protein 4) (31,32) and the open reading frame KIAA0338. In *Drosophila*, Coracle, a band 4.1-related protein, is enriched in septate junctions between epithelial cells, which are equivalent to tight junctions in vertebrates (33). Last but not least, CEDEP, a recently cloned protein, contains an N-terminal band 4.1/JEF domain closely related to that of band 4.1, a Rho-guanine nucleotide exchange factor db1 homology domain, and a PH domain (34). Thus,

band 4.1 and its close relatives are expressed in many cell types in a wide range of organisms.

Potential transmembrane partners of band 4.1-related proteins, such as anion exchanger or CD44, are also widely expressed. Moreover, the presence of a sequence highly similar to the 4.1 binding site of glycophorin C has been identified in an otherwise unrelated glycoprotein, paranodin (35) [also termed contactin-associated protein, caspr (36)]. Paranodin is a neuronal glycoprotein highly enriched in the septate-like junctions between myelinating glial cells and axons on both sides of the Ranvier nodes in the central and peripheral nervous system of mammals. Paranodin binds band 4.1 and other proteins with band 4.1/JEF domains *in vitro* (35 and T. Galvez, L. Goutebroze, J.A. Girault, unpublished results). One probable role of paranodin is to interact with glial proteins on the extracellular side and to provide an anchoring point for the axonal cytoskeleton on the intracellular side, thereby participating in the structure of paranodal junctions. Paranodin is related to a *Drosophila* protein, neurexin IV, located in septate junctions between epithelial cells during embryogenesis and between glial cells (37). Neurexin IV also has a conserved band 4.1-binding sequence and interacts with Coracle, a *Drosophila* relative of band 4.1 (33,38). It has been proposed that other neurexins (I-III), which have some sequence similarity with the band 4.1-binding region of glycophorin C and paranodin (Fig. 4a), might also be band 4.1-binding proteins (39). This is an interesting possibility, which should be experimentally tested, since CASK, a MAGUK protein, is also associated with neurexins (40) and binds to band 4.1 (41). Proteins related to band 4.1 are probably attached to a number of transmembrane proteins with different extracellular domains but with a conserved intracellular segment. A tentative consensus binding site for band 4.1, the GNP motif (Glycophorin C, Neurexin IV, Paranodin), can be built by comparing sequences of the three proteins that have been shown to interact with band 4.1 (Fig. 4a). Using the BLAST screening program (42), we were able to find a similar motif in the membrane-proximal C-terminal region of several novel transmembrane proteins (Fig. 4a). These proteins are strong candidates for binding band 4.1 or related proteins.

(A) Glycophorin C, neurexin IV, paranodin: the GNP motif

Glycophorin C a₆₇ avaiivlsllfvm lRyMYRHKG YHNEAKGT-FAESADAAALQGD-----ALQDAGDSSRK-EYFI
 Neurexin IV v₁₂₂₄ ilflllllmffliGRYLHRHKGDTLEDGA-DGADDDDAVLHSTTG--HQV-----RKRTEFI
 Paranodin v₁₂₉₁ aflllglvglmvlfyIQNHRKGYHTNEPKATHD SHPGGKAPLPPSGPAQAPAPTPAPTPAQVPTPAPAPASGGPGP₁₃₆₆

GNP motif ryxhR⁺hK⁺sYhT⁺bExBgtxd

B12 a₂₃₅ vvvfamcllllllGRYFARHKGYFTFAKGDAAAD-ADTAIINAGQNSEE-----KK-EYFI
 F22162.1 a₃₂₄ llvfllicvlgmvvCSVRQKGYLT EASCLDEQGE-AREAFLNGSDGH-----KRKEEFI
 CD46 i₃₃₉ vvgvavicvvpvrylQRKKGKGYLTDETHREVKFTS
 CET01G9.3 l₅₁₅ faaillmiigciyfirerQYKGYVTRHRSRTPLTMTANTHSCSSSTNDTHGPLSPFPDFLVSSTETFKATPELIPAPPKPGSSVFGI

NRX I- α a₁₄₃₉ aaaaalciillyamyKYRNDEG⁺YHVE⁺SRN⁺YISNSAQSN⁺GAVVKEKQ⁺FSSAKS⁺ANKN⁺KK⁺DK⁺EY⁺YV
 NRX II- α a₁₆₄₇ aaaaalciillyamyKYRNDEG⁺YQVD⁺SRN⁺YISNSAQSN⁺GAVVKEKAP⁺AAK⁺TPSKAK⁺KN⁺DK⁺EY⁺YV
 NRX III- α a₁₅₁₁ aaaaalciillyamyKYRNDEG⁺YQVD⁺TRN⁺YISNSAQSN⁺GTL⁺MKEK⁺QASS⁺ KSGH⁺KK⁺QKN⁺DK⁺EY⁺YV

(B) Other ligands of the band 4.1/JEF domain

CD44 l₃₇₇ lalalilavciavNSRRRCGGK⁺KK⁺LVINGNGTVEDR⁺KPSELNGEASK⁺QEMVHLV⁺NKEPSET⁺PDQMTADETRNLQ⁺SDVMKIGV
 CD43 t₂₄₂ vvvlvllvallllwRQ⁺KRR⁺TGALT⁺LSRGGKNGTVDAWAGPARV⁺DEEAT⁺FASGGN⁺KSSGAPET⁺DGSGQRPTLLT⁺TTFFSR⁺RKSR⁺₃₂₇.....
 ICAM-2 f₂₃₇ vtsvllc⁺fiFGQHW⁺RRR⁺TGT⁺YGVLA⁺AWRRLLP⁺RAFRRP

Band 3 A₃₃₁ LLSLVPVQRELLRR⁺RYQSSPAK⁺PDSS⁺FYKGLDLNGG⁺DDPLQQTG⁺QLFGGLV⁺RD⁺IRRR⁺YPYLLSDITDA₄₀₀

P55 R₂₂₇ VASMAQSA⁺PSAPSE⁺APSCSP⁺FG⁺KK⁺KK⁺YK⁺DKYLAK⁺HSSIFDQ₂₆₅

Integrin β 1 a₇₄₆ llllwKLLMI IHDRREFAK⁺FEKEK⁺MNAK⁺WDTGEN⁺PIYKSA⁺VTVV⁺NPKYEGK
 Integrin β 2 l₇₁₈ llviwKALIIHLSDLREYR⁺RF⁺EKEK⁺LKSQ⁺WNN⁺NDN⁺PLFKS⁺ATTTVM⁺NPKFAES
 Integrin β 3 a₇₃₆ alliwKLLITIIHDRKEFA⁺KFEERARAK⁺WDTAN⁺NPLYKEAT⁺STFTNITYRGT

Fig. 4. Sequence of some identified partners of band 4.1/JEF domains. (A) The sequence of glycophorin C necessary and sufficient for binding band 4.1 has been defined (21,22) (underlined). *Drosophila* neurexin IV binds Coracle (38) and rat paranodin binds band 4.1-related proteins (35). Conserved residues between these three proteins define a GNP motif that was found in several proteins or open reading frames by BLAST 2 analysis, using the intracellular domain of glycophorin C, neurexin IV, or paranodin as queries. Neurexins I-III, which have a related, albeit less conserved sequence, have also been proposed to be band 4.1 ligands (39). Residues identical in the proteins of the GNP group are white on black background (conserved in the three proteins) or gray background (conserved in two proteins). Similar residues are black on light gray background. The conserved motif at the C terminus of several of these proteins is not part of the GNP motif but

is a proven or potential binding site for PDZ domains. Accession numbers: human glycophorin C: P04921; *Drosophila* neurexin IV: AF039833; rat paranodin: AF000114; mouse B12: AF061260; human F22162.1: AC005525; human CD46: 1708964; CET01G9.3: Z75713; rat neurexins (NRX): I- α , A40228; II- α , C40228; III- α , L14851. (B) Other ligands of band 4.1/JEF domains include CD44, CD43, and ICAM-2 (47) for ERM proteins, band 3 (25) and p55 for band 4.1 (21), and β integrins cytoplasmic tail for FAK (100). Sequences shown to be important for binding in the references are underlined. Clusters of basic residues are in boldface. Accession numbers: Mouse CD44: X66081; rat CD43: P13838; human ICAM-2: X65490; human band 3 anion exchange protein 1: P02730; human p55: M64925; human integrins: β 1, P05556; β 2, 825636; β 3, P05106.

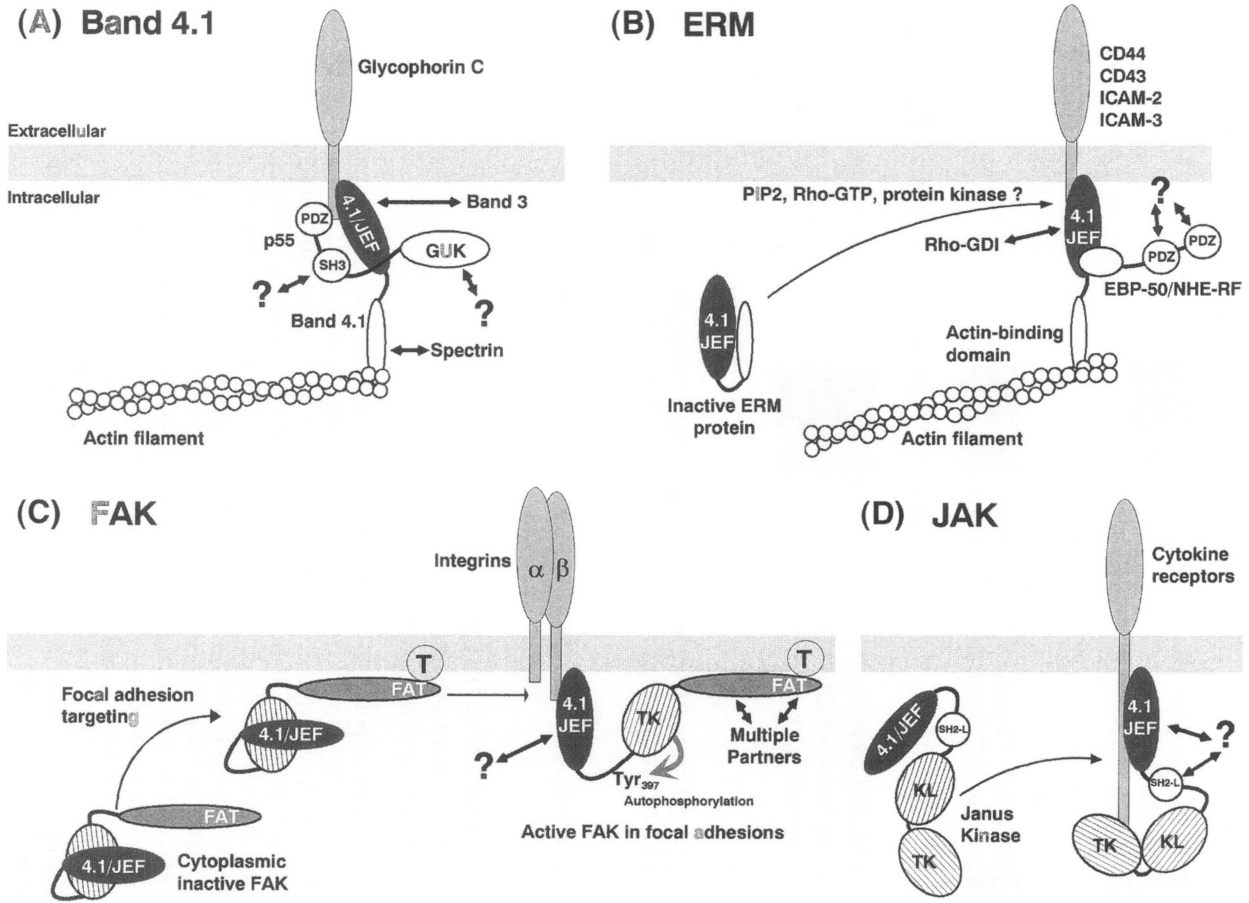


Fig. 5. Functional interactions of band 4.1/JEF domains with transmembrane proteins. The interactions of four well-studied examples of proteins containing band 4.1/JEF domain with transmembrane proteins are schematized. (A) In erythrocytes band 4.1 binds to the cytoplasmic tail of glycophorin C and with a specific sequence of p55, a protein that also contains PDZ and SH3 domains. The PDZ domain of p55 binds to the C terminus of glycophorin C, forming a ternary complex. Other partners of band 4.1 include actin, spectrin, and band 3. (B) ERM proteins are thought to be in a closed state and, following activation, to bind to the cytoplasmic tail of several transmembrane proteins (CD44, CD43, ICAM-2, ICAM-3). This activating step may involve binding to phosphatidylinositol 4,5-bisphosphate (PIP2) and action of Rho-GTP and/or a protein kinase. ERM proteins also interact with EBP-50/NHE-RF, a protein with two PDZ domains associated with Na⁺/H⁺ exchanger, and with

Rho-GDI. (C) FAK is in the cytoplasm in an inactive state. Following integrin engagement, it is targeted to the focal adhesions by a C-terminal sequence (FAT, focal adhesion targeting) that interacts with an unidentified target (T) and multiple other proteins. The band 4.1/JEF domain interacts with the cytoplasmic tail of β integrins, and this may participate in the activation of FAK. Although for the sake of simplicity the autophosphorylation of FAK is shown as an intramolecular reaction, it may be intermolecular. (D) The JAK kinases interact with the membrane-proximal region of cytokine receptors (only one chain is shown), through their N-terminal band 4.1/JEF domain. Activation of the kinase requires additional steps triggered by cytokine binding to the receptor (not shown). GUK, guanylate kinase domain; TK, tyrosine kinase domain; KL, kinase-like domain. Question marks (?) indicate the likely existence of proteic partners, yet to be identified.

The ERM Proteins

Ezrin, radixin, and moesin are three related proteins of ~80 kDa that have an N-terminal band 4.1/JEF domain and the ability to bind actin (10,11,43). Although there are some sequence differences in their C-terminal moiety (e.g., ab-

sence of a polyproline stretch in moesin) and in their pattern of expression, the ERM proteins share a number of properties. Like band 4.1 they appear to function as cross-linkers between the actin cytoskeleton and the plasma membrane, and are enriched in microvilli, filopodia, mem-

brane ruffles, and cell contacts (10,11,43). They bind actin through their C terminus and, possibly, two additional sites, at the N terminus and in the band 4.1 domain (res. 281–333) (44). The ERM proteins can interact with CD44, a large and ubiquitous transmembrane glycoprotein, which acts as a receptor for hyaluronic acid, collagen, fibronectin, and serglycin (45) (Fig. 5b). ERM proteins can bind to other transmembrane proteins that have a more restricted pattern of expression such as CD43 and intercellular cell adhesion molecules (ICAM) 2 and 3 (46,47). ERM proteins also co-localize with the H^+/K^+ ATPase and are associated with EBP-50/NHE-RF, a protein with two PDZ domains that regulates the Na^+/H^+ exchanger (48,49). In addition, ERM proteins bind to Rho-GDI and reduce its activity, thereby initiating the activation of Rho (50). This observation may be related to the requirement of ERM proteins for the Rho-induced stress fibers formation (51).

ERM proteins have a long α -helical region with the ability to form coiled-coil interactions and regions of intramolecular recognition in the N and C termini (52,53). These features lead to intra- or intermolecular head-to-tail interactions that are thought to maintain the ERM proteins in a closed conformation as inactive monomers or to provide the basis for dimerization (11,43). Several regulatory factors contributing to the activation of ERM proteins have been reported. ERM proteins contain a conserved tyrosine phosphorylation site that might be involved in the regulation of oligomerization (54). PIP2 binds to the N-terminal band 4.1 domain of ERM proteins and promotes their interaction with CD44 in vitro (55,56). The action of Rho-GTP is necessary for the binding of ERM proteins to CD44 (56). One possible mechanism of action of Rho is the activation of Rho-kinase and the phosphorylation of ERM proteins on a conserved C-terminal threonine, which prevents the head-to-tail interaction (57) (Fig. 5b).

Schwannomin/Merlin

The gene mutated in neurofibromatosis type 2 (NF2) codes for a tumor suppressor termed schwannomin (58) or merlin (*moesin-ezrin-radixin-like protein*) (59) that has a high degree of sequence identity with ERM proteins (43% overall identity with ezrin, 62% in the band 4.1 N-terminal domain). Schwannomin is co-localized with cortical actin (60–62) and is enriched in membrane ruffles (63). Although it lacks the

C-terminal actin-binding domain of ERM proteins, schwannomin may bind actin by its N-terminal domain (res. 178–367) (64) and one of its splice isoforms (isoform 2) interacts with spectrin by its C-terminal domain (65). Schwannomin also interacts with CD44 (60), microtubules (64), and EBP-50 (48). In *Drosophila*, homologues of moesin (D-moesin) and merlin/schwannomin (D-merlin) are both associated with the plasma membrane but the latter appears to be co-localized with the endocytic compartment (66). The precise mechanism by which schwannomin and D-merlin exert their anti-proliferative effects is not known. Loss of function of D-merlin increases cell proliferation and it was demonstrated that all its essential genetic functions reside in its N-terminal 350 amino acids, corresponding to the band 4.1/JEF domain (67). The consequences of NF2 loss of heterozygosity are severe in transgenic mice, leading to the occurrence of a range of highly metastatic tumors (68). Thus, defects in schwannomin and perhaps in other members of the ERM family have an important role in tumor formation and metastasis. Interestingly, Expanded, a *Drosophila* protein with a divergent band 4.1/JEF domain (Fig. 3) enriched in adherens junctions, also regulates cell proliferation (69). These molecules, like other tumor suppressors associated with cell–cell junctions, such as APC and dlg (70), may control cell proliferation in response to clues provided by cell contacts. It is likely that such controls are very important in regulating cell proliferation during organ development, as suggested by the phenotypes resulting from their mutation in *Drosophila* (67,69).

Talin and Motor Proteins

Band 4.1/JEF domains are found in several large proteins closely associated with cytoskeleton. One is talin, a 270-kDa cytoskeletal protein enriched in focal adhesions (71). Talin has multiple actin-binding sites and a band 4.1 domain located near its N terminus. Talin can dimerize and interact with a number of proteins including β -integrin cytoplasmic tail, vinculin, and FAK (71,72). Band 4.1/JEF domains are also found in specific isoforms of motor proteins, belonging to the myosin and kinesin groups, that are associated with actin filaments and microtubules, respectively. Myosins VIIa and X are unconventional myosins containing band 4.1/JEF domains located at the C termini (Fig. 1) (73,74). The presence of band 4.1 domains might give these

proteins a mode of membrane association unique among the unconventional myosins. In addition, myosin X contains PH domains, which may also contribute to their membrane attachment. Myosin VIIa is enriched in stereocilia of cochlear and vestibular sensory cells and is necessary for the integrity and function of these cells (74). Band 4.1/JEF domains are also found in a specific group of microtubule-associated proteins related to the kinesin family in plants. Kinesin-like proteins containing band 4.1/JEF domains cloned in *Arabidopsis thaliana*, *Nicotiana tabacum*, and *Solanum tuberosum* have the unique property of binding the Ca^{2+} -calmodulin complex and are termed kinesin-like calmodulin binding proteins (KCBP) (75). These proteins appear to have a role during cell division (76). It can be speculated that the presence of a band 4.1/JEF domain in unconventional myosins VIIa and X and in (KCBP) may allow their direct association with specific transmembrane receptors, resulting in the movement of membranes relative to actin- or microtubule-based cytoskeleton.

Protein Tyrosine Phosphatases

Three groups of large protein tyrosine phosphatases (PTPs) contain band 4.1/JEF domains. They all have an N-terminal 4.1 domain and a C-terminal phosphatase domain but their sequences are unrelated between these two domains. These PTPs have been cloned independently by several laboratories and have received different names. One group, characterized by a central PDZ domain, includes two different enzymes: PTPN_3 [PTP H1 (77)] and PTPN_4 [PTP MEG (78)]. The second group also consists of two different enzymes, PTPN_E [PTP36 (79) or PTP-PEZ (80)] and PTPN_F [PTP-D1 (81) or PTP-RL10 (82) or PTP2E (83)], that have a similar structure but no PDZ domain. The sequences of the band 4.1/JEF domain of these PTPs are closely related to those of protein 4.1.

In contrast, the band 4.1/JEF domain of a third group of PTP is more divergent (Fig. 3). These phosphatases, known as PTP-BAS (84), PTP-BL (85), PTPIE (86), PTPL1 (87), or FAP-1 (88), are very large proteins of >2400 residues with five central PDZ domains. The human phosphatase FAP-1 interacts with the C terminus of Fas by two of its PDZ domains (PDZ 2 and 4) (88,89). However, the significance of this observation is unclear (90). In general, little is known about the function and regulation of the PTPs with band 4.1/JEF domains. It is likely that the

presence of a band 4.1/JEF domain allows their targeting to specific cell locations by its association with specific membrane proteins. This could result in the enrichment of PTPs in the vicinity of areas of cell contacts and/or in association with cortical cytoskeleton. However, the precise intracellular localization of these enzymes has not been determined.

The FAK Family

Focal adhesion kinase is a nonreceptor tyrosine kinase substrate for Src and is enriched in focal adhesions (91,92). The knockout of FAK gene is embryonic lethal (93). Its role is thought to include signaling from integrins, turnover of focal adhesion, anti-apoptotic effects of cell contacts with the extracellular matrix, and plasticity in the adult nervous system (94–96). FAK is autophosphorylated in response to integrin engagement or stimulation of a number of G protein-coupled receptors (97). The autophosphorylated tyrosine-397 binds with high affinity Src family kinases that in turn phosphorylate other residues and neighboring proteins, leading to the activation of multiple signaling pathways (98). FAK consists of a central tyrosine kinase domain flanked by a C-terminal domain, which is the site of multiple protein-protein interactions, and an N-terminal domain whose similarity with band 4.1 domain was recently demonstrated (15,16). The region located close to the C terminus is necessary for the targeting of FAK to focal adhesions (focal adhesion targeting, FAT) (99). Although it does not seem to be involved in this targeting, the N-terminal domain of FAK interacts *in vitro* with β integrins (100).

Autophosphorylation of FAK on Tyr-397, which is located between the band 4.1/JEF domain and the kinase domain, is a critical step in its activation. However, the precise mechanism of this autophosphorylation is not known. It might involve a transient dimerization and an intermolecular phosphorylation, by analogy with growth factor receptors. Alternatively, it could result from the suppression of an intramolecular inhibition, as in the case of Src-family kinases, or from a combination of the two mechanisms (i.e., suppression of intramolecular inhibition leading to transphosphorylation). Accordingly, deletion of the region corresponding to the band 4.1/JEF domain increases FAK autophosphorylation *in vitro* (101,102). Moreover, permanent membrane association by fusion of the N terminus with a transmembrane protein results

in a constitutive activation of FAK (101). These observations are compatible with an intramolecular inhibition by the N-terminal domain (96). Interestingly, neuronal splice isoforms of FAK, which differ from the ubiquitous form by the insertion of short peptides on both sides of tyrosine-397, have a dramatically increased autophosphorylation, suggesting that slight modifications in the peptide joining the kinase domain and the band 4.1/JEF domain and containing the autophosphorylation site have important consequences for activation of the enzyme (103). Thus, FAK is targeted to focal adhesions by its C-terminal region, whereas the N-terminal band 4.1/JEF domain may contribute to its regulation by interacting with integrins or other as-yet unidentified molecules (Fig. 5c).

To date, the only known tyrosine kinase closely related to FAK is proline rich-tyrosine kinase 2 (PYK2) (104), also named cell adhesion kinase β (CAK β) (105), related adhesion focal tyrosine kinase (RAFTK) (106), Ca²⁺-dependent tyrosine kinase (CADTK) (107), or FAK2 (108). PYK2 displays a high degree of sequence similarity with FAK over most of its sequence. However, the focal targeting properties of PYK2 appear to be repressed in most circumstances (109). Src-family kinases interact with PYK2 following its autophosphorylation on a tyrosine residue (Tyr-402) corresponding to the autophosphorylation site of FAK (110). Although the partners and activation of PYK2 have not been as extensively studied as those of FAK, there are striking similarities and some differences between the two kinases (96). One characteristic of PYK2 is its sensitivity to increased free intracellular Ca²⁺ (104,107), at least in part mediated by protein kinase C (111–114). The band 4.1/JEF domain most closely related to that of FAK and PYK2 is found in KIAA0316, a predicted open reading frame that contains an N-terminal PDZ domain and a long C-terminal region of unknown function (Figs. 1 and 3; see above).

The JAK Family

The JAK tyrosine kinases are an important group of nonreceptor tyrosine kinases that associate with the intracytoplasmic region of receptors for a number of cytokines and polypeptidic hormones or growth factors (115,116). These receptors are devoid of intrinsic enzymatic activity, but form homo- or heterooligomers that associate with tyrosine kinases of the JAK family. Each receptor chain interacts

preferentially with one or several JAKs. The signaling cascade initiated by these receptors has been extensively studied (see refs. 115,116 for references). Following binding of the cytokine or hormone to the extracellular domain of the receptor, the JAK tyrosine kinases are activated. They phosphorylate the receptor and recruit the STAT proteins, which are unusual transcription factors with SH2 domains. When phosphorylated on tyrosine by JAKs the STATs dimerize, translocate to the nucleus, and activate transcription of specific genes.

The JAKs contain a number of regions in which the residues are conserved among all the members of the JAK family. These regions have been termed JAK-homology regions (JH) and are numbered 1–7, starting from the C terminus (by analogy with the SH domains of the Src family) (115,116). JH1 is the tyrosine kinase domain. JH2 is a kinase-like domain that appears to be important for the function of the JAKs but whose precise role is not known. The N-terminal half of the molecule comprising JH3–7 is far less characterized. The region surrounding JH3 has been shown to possess the structural features of a SH2 domain (117). However, no ligand for this SH2-like domain has yet been identified. The N-terminal region of JAKs, corresponding to the band 4.1/Jef domain (15), is responsible for their interaction with the receptors and the specificity of this interaction (115,116) (Fig. 5d). Interestingly, the N-terminal limit of the region of Tyk2 necessary for interaction with the interferon α/β receptor (118) corresponds to the limit of the band 4.1/JEF domain. Thus, the N-terminal half of JAKs contains two well-defined globular domains, a band 4.1/JEF domain, and a SH2-like domain. The most N-terminal region (corresponding to the first subdomain of band 4.1/JEF domain and the hinge region, see above) is primarily involved in the binding to the receptor (118). The sequences in the receptor that interact with the JAKs have not been fully identified. The membrane-proximal region is critical for the binding of JAKs and a short proline-rich sequence (Box 1) appears to contribute to the specification of the JAK isoform that interacts with the receptor (115,119–121). However, little is known about the regulation of the JAK-receptor interaction and their other partners. Interestingly, one report shows the possibility of an association between JAK2 and FAK (122).

Common Themes among Band 4.1/JEF Domains

This brief overview of the proteins containing band 4.1/JEF domains and of their properties allows us to identify a number of common themes. Obviously, the existence of structural similarities with a low level of sequence identity does not imply that all the functional properties are identical within the superfamily. On the contrary, significant divergence and specialization of the domains are expected. However, it is also likely that some features may be conserved in many members of the superfamily. Thus, information on the most-studied proteins with these domains can be used to generate interesting working hypotheses for the other proteins.

Interactions with the Membrane-Proximal Domain of Transmembrane Proteins

Many of the proteins with band 4.1/JEF domains interact with the cytoplasmic region of proteins having a single transmembrane segment and an intracellular C terminus. In most cases (Fig. 4) as well as in the case of cytokine receptors (see above), the interaction involves the membrane-proximal region of the transmembrane protein. Some of these proteins (glycophorin C, neuexin IV, paranodin) share a short region of similarity, with a GNP consensus motif also found in other proteins which are good candidates for binding proteins with band 4.1/JEF domains (Fig. 4a). Other proteins (CD44, CD43, ICAM-2, band 3) possess stretches of basic residues (also found in p55) that appear to be important for the interaction with band 4.1/JEF domains (Fig. 4b). This suggests that there may be several distinct binding sites on band 4.1/JEF domains. The careful study of the interactions between the various band 4.1/JEF domains and their partners is now required to determine the generality and specificity of these interactions and the structure of the regions involved in binding.

Interactions with Cytoplasmic Proteins

In addition to intrinsic membrane proteins, band 4.1/JEF domains can bind to a variety of peripheral or cytoplasmic proteins. One particularly interesting example is proteins of the MAGUK family which contain PDZ domains. The ternary complex between protein 4.1, p55, and glycophorin C, in which each protein is bound to the two others, has been well characterized in erythrocytes (27). This type of ternary complex can be of

general significance since proteins with PDZ domains, and in some cases guanylate-kinase like domains, are found in association with other proteins which have known or putative binding sites for band 4.1/JEF domains such as neuexins (39–41). Moreover, NHE-RF/EBP-50, a protein interacting with band 4.1/JEF domain of ERMs and schwannomin, possesses PDZ domains associated with Na⁺/H⁺ exchanger (48,49) and several proteins with band 4.1 domains contain PDZ domains themselves (Fig. 1). Many of these proteins have additional interaction domains (e.g., actin-binding sites, spectrin-binding sites, SH3; see above). Thus, band 4.1/JEF domains are likely to participate in multimolecular complexes of potentially unlimited size, forming networks of proteins with multiple interacting domains. Such networks are likely to be the structural framework of many specialized cell regions such as cortical cytoskeleton, focal adhesions, or post-synaptic densities. The exact proteic composition of each network is expected to determine its properties.

Intra- and Intermolecular Interactions of Band 4.1/JEF Domains

In several cases (e.g., ezrin, radixin, moesin, schwannomin, talin) proteins with band 4.1/JEF domains have been shown to undergo intramolecular interactions. These interactions involve one site in the band 4.1/JEF domain and one in the C-terminal region and appear to have a functional importance (53): they allow the formation of homomeric complexes of various sizes (54). They also perform a regulatory role by maintaining the protein in a closed inactive state, until a specific stimulus (e.g., phosphorylation by Rho-kinase in the case of ERM proteins) "opens" the protein (43,45). It will be very interesting to determine if such principles apply to other proteins with band 4.1/JEF domains, including FAK (96). It is also remarkable that heterotypic interactions have been reported between different proteins with band 4.1/JEF domains. For instance, ezrin, radixin, and moesin (54), talin and FAK (72), and JAK and FAK (122) interact with each other. Not all the sites responsible for these interactions have been mapped precisely. However, it is an intriguing possibility that the same regions involved in intramolecular or homotypic intermolecular interactions are also responsible for heterotypic interactions within the group of proteins with band 4.1/JEF domains.

Regulation of the Membrane Association of Band 4.1/JEF Domains

In many cases, the interactions between proteins containing band 4.1/JEF domains and their membrane partners appear to be highly regulated by several factors. The proximity of the membrane may be an important point since binding of a phospholipid (PIP₂) increases the affinity of the band 4.1/JEF domain for its transmembrane partner, as demonstrated in the case of band 4.1 and ERM proteins (13,56). A direct binding of PIP₂ to ezrin has been demonstrated (55), indicating that some of the band 4.1/JEF domains contain a lipid binding site. Binding of PIP₂ may have several important consequences that are not mutually exclusive: it may promote the opening of the protein containing the band 4.1/JEF domain (see above); it may alter the affinity of another binding site for its proteic partner by an allosteric mechanism; and it may provide an additional anchoring point to the membrane.

A second mechanism of regulation of band 4.1/JEF domains binding to the membrane is the intervention of small GTP-binding proteins of the Rho family. Rho-GTP increases the association of ERMs with CD44 (56). Rho is necessary for FAK activation in response to many stimuli (123,124) and Rho-GTP induces the phosphorylation of FAK and associated proteins (125). The mechanism by which Rho exerts its effects is not precisely known. One possibility is that it activates a serine/threonine kinase (Rho-kinase) that phosphorylates ERM proteins' C-terminus, relieving their intramolecular inhibition (57). It has also been suggested that Rho-GTP might activate a phosphatidylinositol-5 kinase, thereby increasing the levels of PIP₂ (45). A strong functional relationship between Rho and some proteins with band 4.1/JEF domains is also indicated by the observation that ERM proteins are required for the effects of Rho-GTP on membrane and cytoskeleton (51). It is noteworthy in this context that the band 4.1/JEF domains of ERMs can associate with Rho-GDI (50) and that CDEP, a novel protein containing a band 4.1/JEF domain, has a domain of homology with Rho-GEF (34).

The properties of proteins with band 4.1/JEF domains can be regulated by phosphorylation. Phosphorylation sites for several serine/threonine or tyrosine kinases have been reported on many of these proteins, and in some cases shown to alter their properties (57,121,126,127). It is possible that different regulatory mechanisms

have evolved for each protein with a band 4.1/JEF domain. However, some properties (e.g., strategic regions for regulation by phosphorylation, presence of a lipid binding site) might be conserved in several, if not all, of these proteins. Determination of the three-dimensional structure of band 4.1/JEF domains will help to address this issue.

Band 4.1/JEF Domains in Multimolecular Complexes Important for Cell Structure and Signal Transduction

Many proteins with band 4.1/JEF domains are enriched at specific intracellular locations, such as cortical cytoskeleton, focal adhesions, adherens junctions, or septate junctions. As discussed above, they participate in multimolecular complexes that are likely to form large networks important for cell structure. Proteins with band 4.1/JEF domains appear to have two types of roles in these complexes. First, they play a structural role by linking transmembrane proteins to actin cytoskeleton (e.g., band 4.1, ERM, talin). In some specific cases (unconventional myosins VIIa or X and KCBP), they may even participate in actin- or microtubule-dependent movements of macromolecules or membranes. A second type of function in which proteins with band 4.1/JEF domains are involved is signal transduction. Several of these proteins have catalytic domains with protein tyrosine kinase or phosphatase activities. The role of the band 4.1/JEF domains may be to target these enzymes to specific cell locations within structural protein networks and to regulate their activity. Thus, they are placed at strategic cellular locations and generate important messages, in response to cell contacts with other cells or with the extracellular matrix. For example, FAK generates antiapoptotic messages in response to cell adherence (128). In addition, several proteins with band 4.1/JEF domains are tumor suppressors (e.g., schwannomin, Expanded), or are closely associated with such proteins (e.g., dlg), whose physiological role is presumably to harmonize cell growth within tissues by controlling the entry into the cell cycle in response to clues coming from neighboring cells. The JAKs constitute a special case among proteins with band 4.1/JEF domains, as they are not enriched at points of contact with other cells or the extracellular matrix and they do not seem to interact with the cytoskeleton. They may represent a divergent utilization of band 4.1/JEF domains during evolution, specialized for the con-

trol of cellular growth in response to secreted polypeptides and not to fixed proteins.

Band 4.1/JEF Domains and Human Pathology

Mutations in proteins with band 4.1/JEF domains play a role in medicine, giving rise to a variety of diseases that underscore the multiplicity of functions in which these proteins are involved. Mutations in band 4.1 gene are responsible for cases of hereditary elliptocytosis with hemolytic anemia (129). The clinical manifestations result from a defect in the cortical cytoskeleton of red blood cells and are similar to those due to mutations in the genes of two proteins associated with band 4.1: spectrin and glycophorin C. A large variety of mutations in the merlin/schwannomin gene are responsible for NF2, which is characterized by the occurrence of multiple schwannomas and meningiomas (130). Somatic mutations of the NF2 gene are also observed in sporadic schwannomas and mesotheliomas (131,132). Schwannomin is absent in many glial tumors as a result of the combination of a germ-line mutation and a somatic mutation in hereditary cases (NF2), or of two somatic mutations in sporadic cases. It is noteworthy that several mutations of merlin/schwannomin identified in human tumors are missense mutations or short deletions in the band 4.1/JEF domain, emphasizing the functional importance of this domain. The mutation of a third protein with band 4.1/JEF domain, myosin VIIa, is responsible for hereditary deafness in mice and humans (74). Mutations in the myosin VIIa gene result in shaker-1 phenotype in mice and in Usher syndrome type 1B in humans. Both diseases include deafness, but patients with Usher syndrome are also blinded by retinitis pigmentosa. It has been hypothesized that myosin VIIa is important for the integrity of the stereocilia of the auditory cells and that it may participate in the phagocytosis of photoreceptors by the retinal pigmented epithelium (133). In the case of JAK family, at least one point mutation occurring in the N-terminal region of JAK3 has been reported in humans, resulting in a severe combined immunodeficiency (134). This mutation (Tyr-100 → Cys) occurs in one of the most conserved stretches of the band 4.1/JEF domain (block 6), underlining its functional importance. Interestingly, a point deletion in the corresponding position in merlin/schwannomin (Phe-96) has been reported in a case of

NF2 (135). By contrast, a point mutation (Gly-341 → Glu) in the *Drosophila* relative to JAKs, *Hopscotch*, which results in a dominant gain of function with leukemia-like hematopoietic defects, is located in a loop that appears to be specific for this protein (136).

Thus, although the superfamily of band 4.1/JEF domains appears to be divergent, the evidence reviewed here suggests that several of their properties have been conserved. We have proposed a number of hypotheses based on this assumption. Experimental approaches, including determination of the three-dimensional structure of representative examples of proteins with this domain, will allow us to test some of these hypotheses.

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