Protein-Protein Interactions in the Tetraspanin Web

Tetraspanins are evolutionarily conserved membrane proteins that tend to associate laterally with one another and to cluster dynamically with numerous partner proteins in membrane microdomains. Consequently, members of this family are involved in the coordination of intracellular and intercellular processes, including signal transduction; cell proliferation, adhesion, and migration; cell fusion; and host-parasite interactions.

**Tetraspanin structure**

As can be inferred from their name, tetraspanins contain four transmembrane (TM) domains. They differ from other proteins with four TM domains by a shared overall structure of the extracellular and intracellular domains and by the presence of conserved amino acid residues (19, 36) (FIGURE 1).

A review focusing on functional domains within this structure has recently been published (50); therefore, the salient features important for protein-protein interactions are briefly summarized here. The tetraspanin TM domains flank a small and a large extracellular loop (SEL and LEL, respectively). The extracellular loops vary among family members. On the other hand, the LEL contains characteristic motifs, especially the conserved cysteine-cysteine-glycine (CCG) motif, which is the focal point for the formation of two to four disulfide bridges with additional cysteine residues at fixed positions within the LEL (45). The three-dimensional structure of the homodimer of one tetraspanin LEL, CD81, has been solved (25, 26) (FIGURE 1). Most tetraspanins’ LELs are glycosylated in one or more potential N-glycosylation sites. Such sites are rarely present in the SEL, and, when they are present, it is unknown whether they are indeed glycosylated. The presence of highly conserved cytoplasmic cysteine residues is an additional hallmark of this family. These cysteines have been shown to be palmitoylated and to play a central role in interactions within the tetraspanin web, as detailed below.

**Tetraspanins are evolutionarily highly conserved and expressed ubiquitously**

The first members of this family were identified in human (22) and in Schistosomes (60). Confirmation of the conservation of this family in evolution comes from recent studies that have identified members of this family in early metazoa (1), in fungi (18), and in nematodes (41). Moreover, multiple tetraspanins are expressed in each organism; for example, there are at least 37 family members expressed in Drosophila (55). In humans, tetraspanins are expressed in all cell types, each of which usually expresses multiple family members.

**Tetraspanins are distinguished from other membrane proteins: clustering in the tetraspanin web**

The most distinctive feature of the tetraspanin family is the ability of its members to form lateral associations with multiple partner proteins and with each other in a dynamic assembly, described as the tetraspanin web (44) (FIGURE 2). The tetraspanin web plays an important role in cell-cell interactions and in cell-fusion events.
Thus CD81 was shown to localize in immune synapses of both B and T cells (38). Both CD9 and CD81 were shown to be involved in egg-sperm fusion (21, 23), and mice that are deficient for CD9 and for CD81 spontaneously develop multinucleated giant cells in the lung (52). The expression of CD81 is required for the infection of hepatocytes by Plasmodium yoelii in mice, and an anti-CD81 MAb inhibits the infection of human hepatocytes by Plasmodium falciparum (48). The tetraspanin PSL1, expressed by the fungus Magnaporthe grisea, is necessary for its binding and penetration of rice leaves (12).

The associations within the web of tetraspanins with their nontetraspanin partner molecules have been referred to as primary (4). A distinct aspect of primary interactions is that a single tetraspanin molecule can form different partnerships in different cell types. Thus the same tetraspanin molecule that forms a partnership with protein X in one cell type can form a partnership with protein Y in a second cell type. For example, CD81 associates in B cells with a B-lineage-specific molecule, CD19 (6), whereas in T cells it associates with CD4 and CD8 (54). The most common partners of tetraspanins are specific members of the integrin (2) and the immunoglobulin superfamilies (6, 49). Finally, partnerships can be formed with the extracellular or the intracellular domains of the interacting molecules. Thus CD81 associates with the extracellular domain of CD19 (5, 37) and with the intracellular domain of another partner, EWI-2 (28, 49). Both CD19 and EWI-2 are members of the immunoglobulin superfamily (FIGURE 3).

Some associations with partners are highly stoichiometric, are highly avid and retained after lysis in harsh detergents (such as 1% NP-40), and are direct, as demonstrated by chemical crosslinking (61, 66). Examples of such avid partnerships are the association of CD151 with α3β1-integrin and the pairing of uropelins (57, 65). Reported additional associations between tetraspanins and partner proteins have been documented by nonquantitative coimmunoprecipitation in milder detergent conditions, such as 1% Brij 96 or 1% Brij 97, or by fluorescent energy-transfer studies (51). However, several detailed studies have demonstrated that specific MAbs, e.g., the anti-CD151 MAb, TS151r (46), and 8C3 (42), can disrupt these associations (24, 42, 46). Therefore, coimmunoprecipitation studies with antibodies that disrupt associations may underestimate the stoichiometry of interactions. Nevertheless, such MAbs are extremely valuable for functional studies. For example, mutating the TS151r binding site in CD151 led to functional

![FIGURE 2. Membrane clustering of tetraspanin-enriched microdomains](image)

A: aerial view of tetraspanin-partner and tetraspanin-tetraspanin interactions. B: side view of lateral associations of tetraspanins with partner molecules. Clustering is facilitated by palmitoylation of conserved cysteines in the intracellular domains of the interacting proteins (right). Tetraspanins are shown in shades of green; partners of the immunoglobulin superfamily are shown in red and brown, and those of the integrin are in blue.
changes in integrin-dependent spreading (24), and the removal of CD151 from α3β1-integrin by the MAb 8C3 led to reduced binding of the stripped integrin to laminin (42).

Tetraspanin-tetraspanin associations, formed between members of the family, have been referred to as secondary (4). These secondary associations are not disrupted by mild detergents (1% Brij 96 or 1% Brij 97) and can be augmented by the presence of divalent cations (7). Tetraspanin-tetraspanin interactions are not stoichiometric, and it is still unknown whether all tetraspanins expressed in a certain cell are associated with each other. Recent studies have demonstrated that palmitoylation is necessary for the maintenance of tetraspanin-tetraspanin interactions. Site-directed mutagenesis of all of the juxtamembrane cysteines of CD9 and of CD151 resulted in the disruption of these secondary interactions (7, 63).

Importantly, tetraspanins associate indirectly with additional proteins, which have been referred to as class 3 interactions. These tertiary interactions are not disrupted in milder detergents, such as 1% CHAPS. Functionally, these interactions cluster in tetraspanin-enriched microdomains, enabling lateral dynamic organization in the membrane and the cross-talk with intracellular signaling and cytoskeletal structures.

**Tetraspanin web proteins cluster in the membrane in tetraspanin-enriched microdomains**

The fact that several tetraspanin molecules form primary, secondary, and tertiary biochemical interactions and that the engagement of individual tetraspanins could lead to identical functional responses in single cell types led to the concept of the tetraspanin web (4). Additional studies documented the clustering of tetraspanins and partner proteins with cholesterol (8) in tetraspanin-enriched microdomain membrane (TEM) (3).

Functional lateral associations of tetraspanins and partner proteins in TEM have been documented in the immune system. For example, antigen-presenting cells (APC) secrete exosomes (17), which are enriched in tetraspanins and were shown to be extremely effective in antigen presentation (70). These exosomes are the secreted fusion products of the plasma membrane with major histocompatibility complex (MHC) class II compartments (MIIC). MIIC consist of multivesicular bodies that are enriched in MHC class II and in the MHC class II-like molecules, which function in the intracellular processing and loading of peptide antigens. In addition, MIIC are enriched in the tetraspanins CD37, CD53, CD63, CD81, and CD82. Tetraspanins were also shown to cluster on the cell surface of APC with a selected subset of MHC class II molecules. In humans, such clusters are identifiable by a specific MAB that interacts only with clustered MHC class II epitopes (CDw78). These clusters were shown to preferentially function in the actual presentation of selected peptide antigens (30).

Tetraspanins also cluster in specialized structural units. For example, there are specialized family members that are present in the apical surface of mammalian bladder epithelium (urothelium) in crystalline structures called urothelial plaques. These plaques are highly enriched in four proteins, two of which, uroplakins Ia and lb (UPIa and UPIb), are tetraspanins that associate with their nontetraspanin partners uroplakin II (UPII) and uroplakin III (UPIII) (67). Biochemical studies have shown that the four uroplakins interact in pairs of one tetraspanin and one nontetraspanin: UPIa/Ii and UPIb/III (33). Uroplakin serves as an excellent model to illustrate how primary interactions are formed because of the reduced complexity of their interactions in the urothelium, as detailed below.

Certain tetraspanins are enriched in specific subcellular organelles. The best example of this is CD63, which contains the lysosomal targeting motif GYEV. Interestingly, the Drosophila sunglasses (sun) tetraspanin, which is most closely related to CD63 and is accumulated in lysosomes, does not contain this motif (62).
Tetraspanin-partner associations during biosynthesis: lessons from knockout mice

The fundamental question of whether tetraspanin-partner associations occur during biosynthesis has recently begun to be addressed. The most stringent dependency on tetraspanin-partner coexpression during biosynthesis has been shown for uropakins. UPIa and UPIb form partnerships with UPIII and UPII, respectively, that are highly stoichiometric. Transfection of uropakin molecules into nonurothelial cells demonstrated that these specific pairings are initiated in the endoplasmic reticulum (ER) and are required for exit of the interacting molecules from this compartment (57). This conclusion was strengthened by the subsequent generation of UPII- and UPIII-deficient mice, which have impaired urothelial plaques because of lack of heterodimer formation (15, 29). These studies in knockout mice establish the role of the non-tetraspanin uropakins (UPIII and UPII) in the exit of their associated tetraspanin uropakins (UPIIa and UPIIb) from the ER/pre-Golgi to the cell surface.

It was therefore expected that CD151, which is strongly associated with the α3-integrin (46, 65) would be required for the cell-surface expression of this integrin. This expectation was strengthened by a study in a human cell line that indicated that CD151 and α3-integrin associate during biosynthesis (24). However, CD151-deficient mice express normal levels of CD19. It is also important to note that the CD81/CD19 partnership differs from that of the paired uropakins in that the relationships are not reciprocal; CD19-deficient mice have normal expression of CD81 (47). Therefore, web assembly may occur in multiple steps in conjunction with trafficking within the different cellular compartments in which web subunits are formed (Figure 4). Tetraspanin-partner interactions may be initiated early, in the ER, as seen for the pairing of uropakins or of CD151 with the α3-integrin. However, these early interactions are not necessarily required for cell-surface expression of the associated tetraspanins and their partner proteins, as seen for CD151 and the α3-integrin. Tetraspanin-tetraspanin associations are more likely to occur in the Golgi compartment because they depend on palmitoylation, as detailed below.

Maintenance and dynamics of interactions in the tetraspanin web

Palmitoylation of the intracellular cysteine residues has been shown to play an important role in the maintenance of tetraspanin-tetraspanin interactions. The replacement of these cysteines by other amino acids, which abolished the palmitoylation of CD9 (7) and of CD151 (3, 63), reduced their association with other tetraspanins. Significantly, the palmitoylation of partner proteins also contributes to their incorporation into TEM, as demonstrated in a recent study of the specific associations of CD151 with the laminin-binding integrins α3β1, α6β1, and α6β4 (64). This study demonstrated that not only CD151 but also its coprecipitated, laminin-binding α3, α6, and β4-integrins, but not α2-integrin, were palmitoylated. Moreover, the replacement of all seven cysteines by serine residues in the β4-integrin abolished the palmitoylation of this integrin. This, in turn, impaired its inclusion in TEM with other laminin-binding integrins, with CD151, and with the additional tetraspanins CD9, CD63, and CD81. Functionally, this depalmitoylation had an effect on cell spreading. The palmitoylation of CD81 was also shown to affect its association with a member of the serine/threonine-binding signaling protein family called 14-3-3s. Interestingly, this association occurred only under oxidative stress, which inhibited the palmitoylation of CD81. This was confirmed by substituting all five cysteines in CD81 with alanines, which induced the association of CD81 with the 14-3-3 protein (11). Most importantly, palmitoylation has been shown to play a key role in the dynamic reorganization of TEM in B cells responding to stimulation. An earlier study demonstrated that coengagement of two complexes on the surface of B cells, the B cell receptor (BCR) complex and the CD19/C21/CD81 complex, reduced the threshold of B cell activation (14). Subsequently, it was demonstrated that the coengagement of the two molecular complexes on the surface of B cells induced membrane reorganization in a CD81-dependent manner, enabling enhanced signaling (10). The most recent study (9) demonstrated that the coengagement of both complexes was accompanied by an increase in the incorporation of labeled palmitic acid into CD81. Moreover, the newly palmitoylated molecules were associated with detergent-resistant microdomains (DRM) and were chased from the low-density sucrose fractions containing the DRM, suggesting that this modification is dynamic.

Tetraspanins as cell-surface receptors

An endogenous soluble ligand, PSG17, has been identified for mouse CD9 (58). The tetraspanin CD9 is required for egg-sperm fusion, and females of CD9-deficient mice have severe fertility impairment (23, 31, 39). Interestingly, the PSG17 ligand...
Tetraspanin–tetraspanin interactions are likely to form in the Golgi upon palmitoylation, serving as building blocks for the assembly of larger multicomponent cell-surface complexes.

Tetraspanins can form specific interactions with their associated partner proteins early in biosynthesis, which serve as building blocks for the assembly of the tetraspanin web. Figure 4

Palmitoylation of specific partners, such as laminin-binding integrins, may also contribute to the clustering in the tetraspanin web. Tetraspanins (shades of green) partner immunoglobulin (red) or integrin (blue) family members. Tetraspanin-partner interactions (blue ties), tetraspanin-tetraspanin interactions (purple ties), and palmitoylation (black-triangled arrows) are also shown.

FIGURE 4. Assembly of the tetraspanin web
Early in biosynthesis, tetraspanins can form specific interactions with their associated partner proteins in the ER, as demonstrated for the pairing of uroplakins and for the association of CD151 with the α3-integrin. Tetraspanin–tetraspanin interactions are likely to form in the Golgi upon palmitoylation, leading to the assembly of larger multicomponent complexes. Palmitoylation of specific partners, such as laminin-binding integrins, may also contribute to the clustering in the tetraspanin web. Tetraspanins (shades of green) partner immunoglobulin (red) or integrin (blue) family members. Tetraspanin-partner interactions (blue ties), tetraspanin-tetraspanin interactions (purple ties), and palmitoylation (black-triangled arrows) are also shown.
CD81 in human cells (34) and by sun receptors were demonstrated to be the cytoskeleton, as detailed in a were identified as cell-surface recep-grin (24). Currently, CD9 and CD81 for their comments on this review.

Its lateral interaction with the homologous region in CD9 is impor-(FIGURE 1 ) (20). Interestingly, the to an epitope located within the inner membranes of the CD9, has been identified as the fusion. An additional tetraspanin, was shown to partially block sperm-fection. This work was supported by the National

We thank Joseph Haimovich and Ronald Levy to coordinate intracellular and inter-

Tetraspanin signaling ports

Additional studies have implicated tetraspanins in the coordination of intracellular signaling pathways with the cytoskeleton, as detailed in a recent review (32). Briefly, Rho-GTPase was shown to link the cytoskeleton with CD82 (13); G protein-coupled receptors were demonstrated to be dynamically regulated by CD9 and CD81 in human cells (34) and by sun in Drosophila melanogaster (62); and phosphatidylinositol-4-kinase and pro-

protein kinase C, the signaling compo-
tents that interact with integrins, were also shown to associate with several tetraspanins (68). In light of these facts, we suggest that TEM are microenvironments analogous to transportation ports. However, unlike seaports and airports, which function as hubs of activity but occupy fixed positions, tetraspanin-signaling ports are dynamic and quick to respond and to coordinate intracellular and inter-cellular activities.

We thank Joseph Haimovich and Ronald Levy for their comments on this review.

This work was supported by the National Institutes of Health grants CA-34233 and AI-45900.

References


3. Berditchevski F, Odintsova E, Sawada S, and Gilbert E. Expression of the palmitoylation-defi-
cient CD151 weakens the association of α6β1 integrin with the tetraspanen-enriched micro-
domains and affects integrin-dependent signa-


6. Bradbury LE, Kansas GS, Levy S, Evans RL, and Tedder TF. The CD19/CD21 signal transdu-


11. Clark KL, Oelke A, Johnson ME, Eilert KD, Auerbach AB, Genieser N, Nelson PK, Robbins EM, and Fearon DT. C3d of complement as a marker, dimerizes with uroplakin Ib as an early stage of melanoma tumor progres-


19. Latorse MP, Pepin R, Tharreau D, Notteghem JL, Henegro J, and Lebrun MH. PLS1, a gene encoding a tetraspanin-like protein, is required for penetra-


27. Kitadokoro K, Ponassi M, Galli G, Petracca R, Falugi F, Grandi G, and Bolognesi M. Subunit association and conformational flexibility in the head subdomain of human CD81 large extrac-


EMERGING TOPICS


