Tetraspanins at a glance

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ABSTRACT

Tetraspanins are a family of proteins with four transmembrane domains that play a role in many aspects of cell biology and physiology; they are also used by several pathogens for infection and regulate cancer progression. Many tetraspanins associate specifically and directly with a limited number of proteins, and also with other tetraspanins, thereby generating a hierarchical network of interactions. Through these interactions, tetraspanins are believed to have a role in cell and membrane compartmentalization. In this Cell Science at a Glance article and the accompanying poster, we describe the basic principles underlying tetraspanin-based assemblies and highlight examples of how tetraspanins regulate the trafficking and function of their partner proteins that are required for the normal development and function of several organs, including, in humans, the eye, the kidney and the immune system.

KEY WORDS: Tetraspanin, Integrin, Membrane compartmentalization

Introduction

Efforts in cloning membrane antigens in the early 1990s led to the cloning of tetraspanin 8 (TSPAN8) and of several clusters of differentiation (CD) proteins, including CD63, CD53, CD37, CD81 and CD9, a then new family of molecules of unknown function (Boucheix and Rubinstein, 2001; Charrin et al., 2009a; Hemler, 2003; Maeker et al., 1997; Yáñez-Mó et al., 2009). These proteins are expressed by all metazoans, with 33 members in mammals, 37 in Drosophila melanogaster and 20 in Caenorhabditis elegans (Huang et al., 2005). Plants also express tetraspanin-like proteins. Tetraspanins have been shown to play a role in many biological processes. Some, such as CD81 and CD37, have an important function in the immune system.
(Jones et al., 2011; Levy, 2014; van Spriel, 2011). Others contribute to the infection by several pathogens (Box 1). Several tetraspanins gained special attention because they regulate the progression of various types of cancers or the formation of metastasis, perhaps as a consequence of their ability to regulate the migration of cancer cells and their ability to invade the surrounding matrix (Boucheix andRubinstein, 2001; Hemler, 2014; Zöller, 2009). In particular, several experimental models, including genetic models of cancers (Copeland et al., 2013a; Copeland et al., 2013b; Deng et al., 2012), as well as clinical data, suggest that CD9 and CD82 are suppressors of metastasis, whereas CD151 and TSPAN8 promote metastasis (Hemler, 2014; Romanska and Berditchevski, 2011; Zöller, 2009).

Despite considerable advances in the understanding of their physiological importance and their implications in several pathologies, the molecular function of tetraspanin proteins remains hypothetical. An increasing number of data, now supported by genetic evidence, show the key role of tetraspanins in regulating the trafficking and function of other membrane proteins and they might do so by regulating the membrane compartmentalization of the molecules they associate with.

Structure of tetraspanins
Tetraspanins are small integral proteins that protrude 3–5 nm from the membrane; they contain four transmembrane domains that delineate two extracellular regions of unequal size and three short intracellular regions (see Poster) (Boucheix andRubinstein, 2001; Charrin et al., 2009a; Hemler, 2003; Yañez-Mó et al., 2009). Structural analysis of the large extracellular domain (LED) of CD81 (Kitadokoro et al., 2001; Rajesh et al., 2012) and molecular modelling (Seigneuret et al., 2001) have provided essential insights into the structure of this domain, which appears to comprise a structurally variable domain inserted into a structurally conserved domain comprising three α helices, one of which immediately follows transmembrane region (TM3) and another of which precedes TM4. This domain is stabilized by disulfide bonds involving four, six or eight cysteine residues. Modelling of the entire molecule (Seigneuret, 2006) suggests that the small extracellular domain fits into a groove of the LED and that the transmembrane regions form a coil-coiled structure, which is stabilized by hydrogen bonds involving the polar residues. Tetraspanins are glycosylated to variable extents and are post-translationally modified through the addition of palmitate moieties on their intracellular cysteine residues (Berditchevski et al., 2002; Charrin et al., 2002; Yang et al., 2002).

Of webs and membrane domains
Tetraspanins interact with one another and with a largely common repertoire of other transmembrane molecules, including integrins and other adhesion receptors, immunoglobulin (Ig)-domain-containing factors, growth factor and cytokine receptors and ectoenzymes (Boucheix andRubinstein, 2001; Charrin et al., 2009a; Hemler, 2003; Yañez-Mó et al., 2009). Most of these interactions are indirect and have been mostly identified by using co-immunoprecipitation with mild detergents that preserve tetraspanin–tetraspanin interactions. The use of some detergents has allowed the identification of directly interacting small tetraspanin–partner complexes (Serru et al., 1999; Yauch et al., 1998). This suggests that various primary complexes assemble through tetraspanin–tetraspanin interactions to form a dynamic network of secondary interactions, we have referred previously to this as the tetraspanin ‘web’ (Boucheix andRubinstein, 2001) (see Poster). Importantly, urothelial plaques assemble on a tetraspanin–partner pair basis (Box 2). Tetraspanin–partner interactions have been shown to involve the LED or transmembrane domains of the tetraspanin (Charrin et al., 2009a; Hemler, 2003; Yañez-Mó et al., 2009). Tetraspanin–tetraspanin interactions are regulated by lipids, including the palmitate moieties that are attached to tetraspanins, membrane cholesterol and gangliosides (Berditchevski et al., 2002; Charrin et al., 2002; Charrin et al., 2003c; Odintsova et al., 2006; Yang et al., 2002). In addition, similar to components of lipid raft microdomains, tetraspanins float to some extent in sucrose gradients after cell lysis (Berditchevski et al., 2002; Charrin et al., 2002; Claas et al., 2001), indicating that they associate with detergent-resistant membranes. These findings have led to the idea that tetraspanins assemble tetraspanin-enriched microdomains (Berditchevski et al., 2002; Hemler, 2003). Importantly, many data suggest that tetraspanins and rafts are different entities (Charrin et al., 2009a; Hemler, 2003; Yañez-Mó et al., 2009), which does not exclude the possibility that these two types of membrane ‘structures’ can cooperate in particular circumstances (Hogue et al., 2011).

Several predictions can be made from the tetraspanin–partner pair model. First, a tetraspanin connects its partner proteins to other tetraspanins and their partners, a prediction that has been validated for interactions between CD151 and integrins, and between CD9 and CD9 partner 1 (CD9P-1, also known as EWI-F and PTGFRN) (Berditchevski et al., 2002; Charrin et al., 2003b;
**Box 2. Specialized tetraspanins**

The tetraspanin uroplakin UPIa and UPIb (also known as UPK1A and UPK1B, respectively) together with two type I integral membrane proteins, UPII and UPIII (also known as UPK2 and UPK3A, respectively), are the major constituents of ‘urothelial plaques’, hexagonally packed 16-nm uroplakin particles that cover the urothelial apical surface. Consistent with the tetraspanin–partner pair model, UPIa and UPIb associate directly with UPII and UPIII, respectively, and are required for the ER exit of their partners (Wu et al., 2009). The 16-nm particles can be resolved into six identical dumbbell-shaped subunits, each comprising an inner and an outer subdomain, which are occupied by one UPIa–UPIII heterodimer and one UPIb–UPIII heterodimer, respectively (Min et al., 2006). Mice that lack the uroplakins UPII and/or UPIII show a loss of apical urothelial plaques and a compromised barrier function of the urothelium. Of note, UPIb serves as the receptor for the uropathogenic type 1-fimbriated *E. coli* (Wu et al., 2009).

The tetraspanins peripherin/RDS (also known as PRPH2) and ROM-1 are two related tetraspanins of the photoreceptor outer segment, which comprises hundreds of stacked membranous disks and is involved in the transduction of light signals into a graded membrane potential. These tetraspanins are highly expressed at the rim of the disks. Mutations in peripherin/RDS are responsible for a broad range of progressive human retinal diseases and, in the mouse, yield dysmorphic outer segments in heterozygous animals (Goldberg, 2006). Peripherin/RDS forms homo-oligomers and hetero-oligomers with ROM-1, which polymerize through intermolecular disulfide bonds involving a seventh cysteine residue that is uniquely present, among tetraspanins, in peripherin/RDS and ROM-1 (Loewen and Molday, 2000). With more than 50 residues, the C-terminal cytoplasmic domains of peripherin/RDS and ROM-1 are, among tetraspanins, exceptionally long. This domain in peripherin/RDS has been suggested to have a fusogenic activity (Boesz-Battaglia et al., 2003) and was recently shown to contain an amphiphatic helix that is able to generate membrane curvature (Khattree et al., 2013), suggesting that there is a direct role of peripherin/RDS in generating the high-curvature bend of the disk rim.

Takeda et al., 2007). Second, tetraspanins can be expected to have a specific role in the regulation of their partners and a non-specific role in contributing to the stoichiometry of all the molecular interactions inside of the tetraspanin web.

**Dynamics inside of the tetraspanin web**

The dynamic behaviour of tetraspanins has been addressed in several recent studies. For instance, analysis of CD9 at the single molecule level using total internal reflection fluorescence (TIRF) microscopy revealed that CD9, at the cell basal surface, cycles between tetraspanin-enriched areas (TEAs) and the rest of the membrane (Espenel et al., 2008). In these enriched areas, it has a confined motion and displays a lower diffusion rate, suggesting that these areas correspond to regions of the membrane to which several molecules are confined at the same time. Elsewhere, CD9 molecules have a Brownian motion. Importantly, TEAs are not equivalent to tetraspanin-enriched microdomains because tetraspanin–tetraspanin interactions occur inside and outside of these structures.

The function of TEAs, which also contain several tetraspanin-associated molecules, is unknown, but TEAs are reminiscent of the endothelial adhesive platforms that form upon the interaction of leucocytes with endothelial cells into which intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) are recruited; several tetraspanins, including CD9 and CD151, are also recruited and display a lower diffusion rate in these platforms (Barreiro et al., 2008). Similarly, the human immunodeficiency virus (HIV) gag protein recruits several tetraspanins at the virus budding site, leading to a confinement of tetraspanins at these sites (Krementsov et al., 2010). Although tetraspanins and their partners can mutually affect the diffusion of each other (Mattila et al., 2013; Potel et al., 2013; Yang et al., 2012), it remains to be determined whether tetraspanins actually recruit their partner proteins into TEAs.

**The intracellular tails of tetraspanins**

Several intracellular proteins have been shown to co-immunoprecipitate with tetraspanins. However, in many cases, for example for phosphatidylinositol 4-kinase (PtdIns4) kinase, activated conventional protein kinase C (PKC) or heterotrimeric G proteins (Berditchevski et al., 1997; Le Naour et al., 2006; Little et al., 2004; Zhang et al., 2001), it is unknown whether these proteins associate directly with a particular tetraspanin. Consistent with the tetraspanin–partner pair model, the PKC-dependent phosphorylation of integrin β4 on serine residues is regulated by CD151 (Deng et al., 2012; Li et al., 2013). By contrast, the C-terminus of CD63 contains a tyrosine-based internalization motif that confers a fast rate of endocytosis, as well as a prominent localization in late endosomes, which is mediated through an interaction with adaptor protein complexes (Berditchevski and Odintsova, 2007; Pols and Klumperman, 2009). This motif overlaps with a PDZ-binding motif that is involved in the binding of CD63 to syntenin, suggesting that, because syntenin has two PDZ domains, some interactions within the tetraspanin web might occur through this (or other) PDZ proteins (Latysheva et al., 2006). Other tetraspanins also have PDZ-binding or potential internalization motifs, but their roles are not as clear as those in CD63 (Berditchevski and Odintsova, 2007). Furthermore, other proteins, such as ezrin and the small GTPase Rac1, have been suggested to interact with the C-terminal domain of CD81 (Sala-Valdés et al., 2006; Tejera et al., 2013), providing a potential link between tetraspanins and the cytoskeleton.

**Tetraspanins that regulate the trafficking of their partner proteins**

There are a few examples of tetraspanins of which the primary function is to control the trafficking of their partner proteins. For instance, a key function of CD63, consistent with the presence of a tyrosine-based internalization motif, might be to facilitate the internalization of discrete partner proteins and/or their targeting to late endocytic organelles (Pols and Klumperman, 2009) (see Poster). Such proteins include synaptotagmin 7, a protein that regulates lysosome exocytosis and membrane repair (Flannery et al., 2010); the stromal cell-derived factor 1 (SDF1) and HIV receptor C-X-C chemokine receptor type 4 (CXCR4) (Yoshida et al., 2008); H’/K’ ATPases (Codina et al., 2005; Duffield et al., 2003); and PMEL17 (also known as PMEL), which is involved in melanogenesis (van Niel et al., 2011). The *Drosophila* tetraspanin Sunglasses (Tsp42Ej) is another example; it is predominantly expressed in the eye and is enriched in lysosomes where it associates with the G-protein-coupled light receptor rhodopsin and regulates its trafficking to lysosomes for degradation. Flies that lack Sunglasses have an impairment in this process that results in light-dependent retinal degeneration (Xu et al., 2004).
Tetraspanins also sometimes (but not always) control the trafficking of their partner proteins in the secretory pathway (see Poster). For example, the metalloprotease ADAM10 is the partner protein of the tetraspanins that have eight cysteine residues in their LED (TSPAN5, TSPAN10, TSPAN14, TSPAN15, TSPAN17 and TSPAN33), referred to collectively as the TspanC8 subgroup. TspanC8 proteins regulate the exit of ADAM10 from the endoplasmic reticulum (ER) and differentially control its targeting to either late endosomes or to the plasma membrane (Dornier et al., 2012; Haining et al., 2012; Prox et al., 2012). Consistent with the key role of ADAM10 in mediating the cleavage of Notch and thus the release of its cytoplasmic domain, which is a transcription cofactor (Hori et al., 2013), certain TspanC8 proteins regulate Notch signalling. Importantly, the TspanC8 proteins are among the few mammalian tetraspanins that have apparent orthologues in *D. melanogaster* and *C. elegans*, which also regulate Notch signalling (Dornier et al., 2012; Dunn et al., 2010). These findings clearly show that specific tetraspanins can influence a signalling pathway by regulating the cell surface expression of their partner protein.

Another example is CD81, which regulates the trafficking of CD19 along the secretory pathway (Shoham et al., 2003). A mutation in CD81 has recently been shown to be the cause of the immunodeficiency of a child displaying a defective humoral response (van Zelm et al., 2010). This mutation causes a strong reduction in the surface levels of CD19, an essential co-stimulatory molecule of lymphoid B cells and a well-characterized CD81 partner, and consequently impaired signalling upon B-cell receptor stimulation. In addition, CD81 can contribute to the activation of B cells through the organization of CD19 nanoclusters (Mattila et al., 2013). CD81 in the mouse also facilitates cognate B-cell–T-cell interactions with an impact on the polarization of helper T cells towards the T helper type 2 profile, which play an essential role in the humoral response (Deng et al., 2002). In this regard, CD81 is concentrated at the immune synapse during B-cell–T-cell cognate interactions and regulates the timing of the maturation of the synapse and signalling in T cells (Rocha-Perugini et al., 2013). Whether this is directly linked to the ability of CD81 and other tetraspanins to interact with CD3, CD4, CD8 or major histocompatibility complex (MHC) class II molecules remains to be determined (Boucheix and Rubinstein, 2001; Levy, 2014).

**Regulation of membrane-anchored enzymes and growth factor signalling**

Tetraspanins regulate the function of a diverse range of membrane proteins. Two recent studies combining biochemical and genetic analyses have highlighted the ability of tetraspanins to regulate the function of associated oligomeric complexes without apparent influence on the trafficking of their partner (see Poster). The first study showed that the *C. elegans* tetraspanin TSP-15 associates with the transmembrane proteins of the dual oxidase (DUOX) pathway (BLI-3 and DOXA-1) and crucially regulates H$_2$O$_2$ production, which is required for collagen cross-linking through tyrosyl residues. As a consequence, worms that lack TSP-15 have an abnormal cuticle, the exoskeleton of the worm (Moribe et al., 2012; Moribe et al., 2004). In the second example, it was found that TSPAN12, but not other tetraspanins, enhances β-catenin signalling in response to norrin (encoded by *NDP*) by promoting the multimerization of its receptor complex, comprising Frizzled-4 and low-density lipoprotein-related protein 5 (Lrp5) co-receptor (Junge et al., 2009). The functional relevance of signalling between TSPAN12 and norrin is strengthened by the finding that alterations in *TSPAN12*, *NDP*, *FZD4* and *LRP5* genes cause similar vascular retinal defects leading to familial exudative vitreoretinopathy in humans (Ye et al., 2010).

Among the membrane proteins that are functionally regulated by tetraspanins, there are several membrane-bound proteases, including ADAM10, ADAM17, MT-MMP1 (also known as MMP14) and the urokinase-type plasminogen activator (Arduise et al., 2008; Bass et al., 2005; Gutiérrez-López et al., 2011; Lafleur et al., 2009; Shiomi et al., 2005; Xu et al., 2009; Yañez-Mó et al., 2008), and several membrane-anchored growth factor precursors, such as transforming growth factor α (TGFα) or heparin-binding EGF-like growth factor (HBEGF) (Higashiyama et al., 1995; Imhof et al., 2008). In addition, several tetraspanins, in particular CD9, CD82 and CD151, associate with and/or modulate downstream tyrosine kinase receptors, including epithelial growth factor receptor (EGFR), ERBB2, c-MET and vascular endothelial growth factor receptor (VEGFR)-3 (also known as FLT4) (Franco et al., 2010; Iwasaki et al., 2013; Murayama et al., 2008; Novitskaya et al., 2014; Odintsova et al., 2003; Sridhar and Miranti, 2006; Takahashi et al., 2007). However, so far, there is no evidence that a particular tetraspanin associates directly with these receptors. By contrast, several studies suggest that the underlying mechanisms, at least for CD82, might be indirect (Odintsova et al., 2006) and that the effect of CD151 on the signalling of these receptors might be a consequence of a functional or physical coupling with laminin-binding integrins (Franco et al., 2010; Novitskaya et al., 2014).

**The regulation of integrin function by CD151 and other tetraspanins is important in adhesion strengthening**

Tetraspanins have long been known to associate with various integrins. Among these, the laminin-binding integrins α3β1, α6β4 directly associate with CD151 (Serru et al., 1999; Yauch et al., 1998) (see Poster). In general, CD151 only minimally affects adhesion or spreading on matrix proteins to which these integrins bind. Instead, CD151 has been shown to regulate the activation of several signalling molecules downstream of these integrins, including Akt, Src, focal adhesion kinase (FAK), p130CAS (also known as BCAR1), paxillin and Rho family GTPases (Hong et al., 2012; Takeda et al., 2007; Yamada et al., 2008; Yang et al., 2008). Importantly, the influence of CD151 on the activation of these molecules appears to depend on the cellular context, suggesting that CD151 does not function as an adaptor molecule for a particular signalling pathway. There is also no indication that CD151 could regulate the binding of soluble laminins to cells. By contrast, several studies point to a role of CD151 in regulating adhesion strengthening (Lammerding et al., 2003; Nishiiuchi et al., 2005; Sachs et al., 2012), a dynamic process that follows integrin-mediated adhesion and involves receptor clustering and interactions with cytoskeletal, structural and signalling elements (Puklin-Faucher and Sheetz, 2009).

Loss of CD151 has a strong effect *in vivo*, both in mouse and human. Particularly dramatic is kidney failure due to glomerular alterations (Baileto et al., 2008; Karamatic Crew et al., 2004; Sachs et al., 2006). Importantly, specific deletion of integrin α3β1 in podocytes causes a similar phenotype (Sachs et al., 2012). The abnormalities that are observed in the absence of CD151 worsen...
with age and are influenced by blood pressure, consistent with a defect in adhesion strengthening in podocytes (Sachs et al., 2012). CD151 can also regulate adhesion strengthening following engagement of the platelet fibrinogen receptor, and integrin αβ3 (Lau et al., 2004) and the tetraspanins CD81 and CD37 regulate adhesion of lymphoid B cells to integrin αβ1 ligands under flow (Feigelson et al., 2003; van Spriel et al., 2012). The impaired activity of integrin αβ1 that is observed in CD37-null B cells is associated with a change in the cell membrane distribution of the integrin and could be the cause of the impairment of Akt-dependent survival of long-lived antibody-secreting cells, which, in turn, leads to impaired IgG1 production in response to T-cell-dependent antigens (van Spriel et al., 2012).

In contrast to many other tetraspanins, CD151 expression has an impact on the growth of primary tumours or tumour onset (Deng et al., 2012; Hemler, 2014; Romanska and Berditchevski, 2011; Zöller, 2009), and this might be owing to its regulation of integrin function. For example, in a genetic model of breast cancer, phosphorylation of FAK has been shown to be diminished in the tumours of CD151-null animals, suggesting a potential defect in integrin signalling. Additionally, data suggest that the reduced susceptibility to two-stage skin carcinogenesis of CD151-null mice is linked to the ability of CD151 to regulate integrins αβ4 or αβ1 (Li et al., 2013; Sachs et al., 2014).

**CD9 and CD81 in cell–cell fusion and other processes: an example of cooperation between two tetraspanins**

CD9 and CD81 are closely related tetraspanins, sharing 45% identity and two common partner proteins – the two related Ig-domain molecules EWI-F/CD9P-1 and EWI-2 (also known as IGSF8) (Charrin et al., 2003a; Charrin et al., 2001; Stipp et al., 2001a; Stipp et al., 2001b). These two tetraspanins have been shown to regulate several cell–cell fusion processes (see Poster). Mouse eggs that lack CD9 are greatly deficient in their ability to fuse with sperm (Kaji et al., 2000; Le Naour et al., 2000; Miyado et al., 2000), and this defect is attenuated by expression of CD81 (Kaji et al., 2002; Rubinstein et al., 2006). The shape of the egg microvilli are also altered in the absence of CD9 (Runge et al., 2007). Furthermore, CD9 and CD81 cooperate in macrophage fusion and muscle cell fusion, but as negative regulators of these processes (Charrin et al., 2013; Takeda et al., 2003). The increased muscle cell fusion observed in their absence has been recapitulated by RNA interference (RNAi)-mediated depletion of CD9P-1, pointing again to the importance of tetraspanin-associated molecules (Charrin et al., 2013). Finally, double CD9 CD81 knockout mice also display a number of pathologies that are not, or only minimally, observed in single-mutant mice, such as pulmonary emphysema (a major pathological component of chronic obstructive pulmonary disease) (Takeda et al., 2008), osteopenia (Takeda et al., 2008) or defects of blood and lymphatic vessels (Iwasaki et al., 2013).

**Conclusions and perspectives**

Recent studies combining genetic and biochemical analyses have highlighted the key roles of distinct tetraspanins in regulating the function and/or trafficking of their partner proteins at the cell membrane. However, there are still a number of functions of tetraspanins for which molecular details are lacking. For example, how do CD9 and CD81 regulate cell–cell fusion? Why does experimental modification of tetraspanin expression levels or the use of antibodies against tetraspanin often induce membrane remodelling (Zhang and Huang, 2012)? How exactly do tetraspanins regulate the immune response, besides the CD81-mediated regulation of CD19 expression? Why are tetraspanins enriched in exosomes (Raposo and Stoorvogel, 2013)? It is likely that a more comprehensive knowledge of the molecular organization of tetraspanins, and in particular the identification of additional tetraspanin–partner pairs, will help to resolve some of these questions. However, some tetraspanins might also play a role by themselves, independently of any protein partner.

The hypothesis that tetraspanins have a role in membrane compartmentalization comes from their ability to interact with many other membrane proteins and their ability to partition into the light fractions of sucrose gradients. Although supported by most of the recent data, this hypothesis still requires a formal demonstration. Super-resolution microscopy techniques and analysis of the dynamics of tetraspanins and of their partner proteins under various conditions are likely to provide essential new insights into the function of these molecules, as these techniques did for the field of lipid microdomains.

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**Competing interests**

The authors declare no competing interests.

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