Biochem. J. (2009) **420**, 133–154 (Printed in Great Britain) doi:10.1042/BJ20082422

multimedia

REVIEW ARTICLE

Lateral organization of membrane proteins: tetraspanins spin their web

Stéphanie CHARRIN*†, François LE NAOUR*†, Olivier SILVIE‡§, Pierre-Emmanuel MILHIET‖¶, Claude BOUCHEIX*† and Eric RUBINSTEIN*†[1]

*Inserm, U602, F-94807, Villejuif, France, †Université Paris-Sud, Institut André Lwoff, F-94807, Villejuif, France, ‡Inserm, U511, F-75013, Paris, France, §Université Pierre et Marie Curie-Paris 6, F-75013, Paris, France, ‖Inserm, U554, F-34090, Montpellier, France, and ¶Université de Montpellier, CNRS, UMR 5048, Centre de Biochimie Structurale, F-34090, Montpellier, France

Despite high expression levels at the plasma membrane or in intracellular vesicles, tetraspanins remain among the most mysterious transmembrane molecules 20 years after their discovery. Several genetic studies in mammals and invertebrates have demonstrated key physiological roles for some of these tetraspanins, in particular in the immune response, sperm–egg fusion, photoreceptor function and the normal function of certain epithelia. Other studies have highlighted their ability to modulate cell migration and metastasis formation. Their role in the propagation of infectious agents has drawn recent attention, with evidence for HIV budding in tetraspanin-enriched plasma membrane domains. Infection of hepatocytic cells by two major pathogens, the hepatitis C virus and the malaria parasite, also requires the tetraspanin CD81. The function of tetraspanins is thought to be linked to their ability to associate with one another and a wealth of other integral proteins, thereby building up an interacting network or 'tetraspanin web'. On the basis of the biochemical dissection of the tetraspanin web and recent analysis of the dynamics of some of its constituents, we propose that tetraspanins tightly regulate transient interactions between a variety of molecules and as such favour the efficient assembly of specialized structures upon proper stimulation.

Key words: CD9, CD81, CD82, CD151, metalloproteinase, tetraspanin.

INTRODUCTION

Tetraspanins comprise a family of integral proteins found in all metazoans [1] of which 33 have been identified in humans and mice (Figure 1). Although other insects have only ~15 tetraspanin genes, *Drosophila melanogaster* has 36, half of which are found in a specific cluster of tetraspanins on chromosome 2 [1–3]. Several tetraspanins have been identified in helminths, 20 in *Caenorhabditis elegans* and at least 25 in the human parasite *Schistosoma* [1], some of which are potential vaccine antigens for use in the prevention of schistosomiasis [4]. Whereas unicellular fungi such as *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* do not express tetraspanins, multicellular fungi do. In particular, a tetraspanin called Pls1 is essential for the infection of rice leaves by the pathogenic fungus *Magnaporthe grisea*, whereas its orthologue plays a key role during ascospore germination in *Podospora anserina* [5–7]. The protozoan parasite *Entamoeba histolytica* expresses at least six tetraspanins; however, none has been identified in two other protozoans, *Leishmania major* and *Plasmodium falciparum*, the latter being responsible for malaria [1]. Finally, plants also express tetraspanin-like proteins, one of which plays a key role in the normal development of *Arabidopsis thaliana* [8,9].

Only a limited number of these molecules have so far been studied in mammals, partly because of the lack of antibodies; however, a large subset of these seem to be expressed in all cell types. Several tetraspanins such as CD9, CD81 and CD151 have a large, although not ubiquitous, cell and tissue distribution. Others have a more restricted pattern of expression. This is particularly the case for tetraspanins belonging to specialized structures, such as the uroplakins UPIa and UPIb constituting the asymmetric unit membranes of the urothelium [10], and the proteins peripherin/RDS and Rom-1 found in the rim of the photoreceptor outer segment disc [11]. CD53 is expressed almost exclusively by leucocytes, and CD37 is expressed by lymphoid T- and B-cells.

Predominantly localized in the endosome/lysosome compartments, CD63 is unique among the well-described tetraspanins in terms of its cellular distribution. Whereas the localization of other tetraspanins studied so far seems to be principally at the cell surface, some are also present in these CD63-positive structures [12]. In macrophages, tetraspanins such as CD9, CD53 and CD81 are present in CD63-negative intracellular plasma membrane domains [13]. Finally, tetraspanins are enriched in exosomes, extracellular vesicles formed in multivesicular bodies, and released upon fusion of these vesicles with the plasma membrane [14].

Abbreviations used: ADAM, a disintegrin and metalloproteinase; AP, adaptor protein; BCR, B-cell receptor; CHO, Chinese-hamster ovary; DARC, Duffy antigen receptor for chemokines; DRM, detergent-resistant membrane; EBP50, ERM (ezrin/radixin/moesin)-binding phosphoprotein 50; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; ERM, ezrin/radixin/moesin; FAK, focal adhesion kinase; FCS, fluorescence correlation spectroscopy; FRAP, fluorescence recovery after photobleaching; FRET, fluorescence resonance energy transfer; GPCR, G-protein-coupled receptor; GPI, glycosylphosphatidylinositol; HB-EGF, heparin-binding epidermal growth factor-like growth factor; HCV, hepatitis C virus; ICAM-1, intercellular cell adhesion molecule 1; IL, interleukin; Ibm, late bloomer; LED, large extracellular domain; mAb, monoclonal antibody; MβCD, methyl-β-cyclodextrin; MMP, matrix metalloproteinase; MT1-MMP, membrane-type matrix metalloproteinase 1; NK, natural killer; p130^{Cas}, Crk-associated substrate; PI3K, phosphoinositide 3-kinase; PI4K, phosphoinositide 4-kinase; PKC, protein kinase C; PSG, pregnancy-specific glycoprotein; TGFα, transforming growth factor α; TIMP, tissue inhibitor of metalloproteinases; TIRF, total internal reflection fluorescence; TM, transmembrane domain; UP, uroplakin; VCAM-1, vascular cell adhesion molecule 1.

[1] To whom correspondence should be addressed (email eric.rubinstein@inserm.fr).

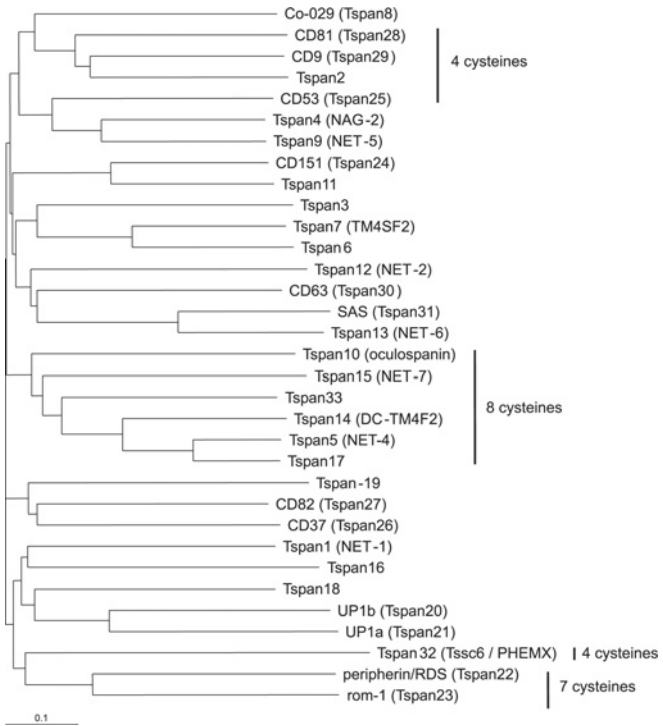

Figure 1 Relative distance of the different human tetraspanins

The sequences of the 33 human tetraspanins were aligned, and a phylogram was generated using the ClustalW program. The number of cysteine residues within the LED is indicated on the right for those tetraspanins that do not have six cysteine residues.

STRUCTURE OF TETRASPANINS

Of the proteins with four TMs (transmembrane domains), tetraspanins can be distinguished by their fairly small size, ranging from 204 (Tspan-13/NET-6) to 355 (Tspan-10/oculospanin) amino acids, and two extracellular domains of unequal sizes (Figure 2) [12,15–17]. The first and smaller domain consists of less than 30 amino acids in the human proteins, whereas the LED (large extracellular domain) contains 76–131 amino acids and protrudes just a few nanometres from the membrane (3.5 nm for CD81, 5 nm for UPIa and UPIb [18,19]). By comparison, the ectodomain of CD2, a molecule with two Ig domains, is 7 nm [20]. The N- and C-terminal tails are cytoplasmic and generally contain less than 20 residues. Tetraspanins can be subjected to two major post-translational modifications, with most (one notable exception being CD81) containing potential N-glycosylation sites (for a review, see [12]) and all those studied so far undergoing palmitoylation [21–26]. This latter process typically involves the covalent attachment of palmitate to cysteine residues located near the interface between the membrane and the cytosol. Tetraspanins have several of such cysteine residues, including six in CD9, CD81 and CD151, and five in CD82, with most, if not all, of them being sites of palmitate attachment [21–24]. In addition, several tetraspanins were very recently shown to undergo ubiquitination [27,28].

The crystal structure of the CD81 LED has been solved [18]. Two antiparallel α-helices (A and E) in the continuity of TM3 and TM4 form the stalk of the domain (Figure 2). The A helix is connected via a short loop with a third helix referred to as the B helix. Molecular modelling indicates that these three helices, although not conserved at the amino acid level, form a structure that is common to all tetraspanins [29]. In CD81, two additional

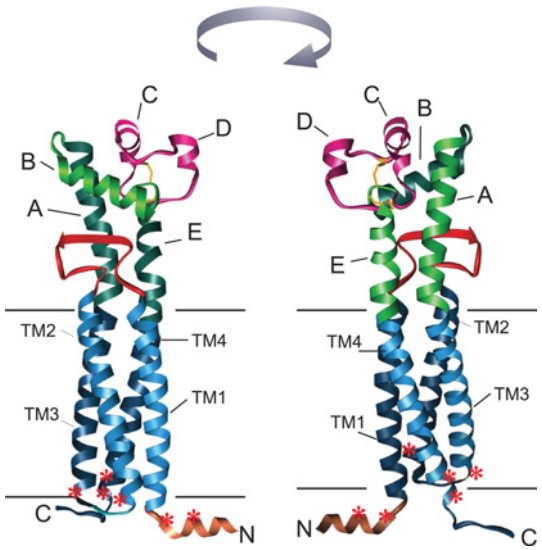

Figure 2 Two different views of the structure of the tetraspanin CD81 according to the modelling of Seigneuret [30] (PDB code 2AVZ)

The transmembrane regions are in blue, the small extracellular domain is in red, the structurally conserved domain of the LED is in green, and the variable region of this domain in deep pink. The disulfide bridges of the LED are in yellow, and the intracellular cysteine residues that are probable sites of palmitate attachment are indicated by red asterisks. The variable region of the LED contains two (including for CD81 as shown here), three or four segments separated by cysteine residues. The different transmembrane regions are indicated (TM1–TM4) as well as the different subdomains of the LED (A–E).

helices (C and D) inserted into the stalk, together with the B helix, form the head subdomain of the LED. This C and D region, found also in other tetraspanins, is not structurally conserved and is therefore referred to as the variable subdomain of the LED [29]. Several conserved cysteine residues, including a CCG motif located after the B helix (a hallmark of tetraspanins) and other residues between the C and D helices and at the beginning of the E helix, contribute towards maintaining the LED structure. Whereas most tetraspanins have six cysteine residues in the LED (and an additional region inserted between C and D), CD81, CD9, CD53, Tspan-2 and Tspan-32 have only four and others have eight (see Figure 1). The retinal protein peripherin/RDS and Rom-1 have a seventh cysteine residue responsible for the formation of higher-order polymers [11]. The variable domain containing the C and D helices lies opposite a hydrophobic patch present in several tetraspanins which could be involved in the packing of a β-strand present in the small extracellular loop [19,30].

The majority of the conserved residues are located in the TMs. Modelling of these domains suggests that they are left-handed coiled coils and that many of the conserved residues are involved in the packing of the helical bundles, allowing either typical 'knob in the hole' interactions (for small and large hydrophobic residues) or hydrogen-bonding (for polar residues) [30,31].

TETRASPANINS AS RECEPTORS

Until recently, no physiological ligands had been identified for tetraspanins. Now however, PSG (pregnancy-specific glycoprotein) 17, a ligand of CD9, has been characterized. This molecule belongs to the CEA (carcinoembryonic antigen) subfamily of the immunoglobulin superfamily. Binding of the mouse molecule to macrophages, as well as its consequential ability to induce the production of anti-inflammatory cytokines, requires CD9 [32]. A direct interaction between PSG17 and CD9,

requiring the LED residue Phe[174] in the D region of CD9, has been demonstrated using recombinant proteins [33]. PSG17 is produced exclusively by the placenta, and its presence in the circulation of pregnant women may play a role in the generation of an immune environment compatible with successful pregnancy [34].

CD9 may also be a receptor for the pro-inflammatory cytokine IL (interleukin)-16. Indeed, antisense CD9 oligonucleotides were shown to strongly decrease and anti-CD9 mAb (monoclonal antibody) block the binding of this cytokine to a human mast cell line. Conversely, expression of CD9 in CHO (Chinese-hamster ovary) cells induces IL-16 binding and responsiveness in terms of calcium mobilization [35].

TIMPs (tissue inhibitors of metalloproteinases) regulate the dynamic processes of extracellular matrix turnover and remodelling, as well as the activities of growth factors and their cell-surface receptors, in part via the inhibition of MMPs (matrix metalloproteinases). Metalloproteinase-independent effects of TIMPs have also been reported [36]. Screening of a human placental library using the yeast two-hybrid system and full-length TIMP-1 cDNA as bait revealed CD63 as a binding partner. Immunoprecipitation assays confirmed this interaction (whether TIMP-1 was ectopically expressed in cells or added exogenously), whereas no interaction was detected between CD63 and TIMP-2. Knockdown of CD63 strongly reduced the binding of TIMP-1 to cells and prevented some TIMP-1-mediated effects such as the activation of ERK (extracellular-signal-regulated kinase) and protection from apoptosis [37]. In addition, using a similar approach, Shiomi et al. [38] found that CD151 could bind to pro-MMP-7.

Another example of a potential tetraspanin ligand is DARC (Duffy antigen receptor for chemokines), identified as a CD82-binding molecule after screening a human normal prostate library using the yeast two-hybrid system and the full-length CD82 cDNA as bait [39]. Expressed by endothelial cells and erythrocytes, this seven-transmembrane protein plays a role in the clearance of chemokines following binding without triggering signal transduction. We will see below how DARC may be involved together with CD82 in the suppression of metastasis.

It should be kept in mind that the definitive demonstration of a protein as a ligand for a tetraspanin is difficult. In this regard, the CD9/HB-EGF (heparin-binding epidermal growth factor-like growth factor) story provides a good example. Indeed, an anti-CD9 mAb was initially screened on its ability to block the binding of diphtheria toxin to cells and CD9 was subsequently found to increase toxin binding and cell sensitivity. However, it turned out that the toxin receptor was, in fact, the membrane-anchored precursor of HB-EGF that associates with CD9 (see [40] and references within). This interaction is only one of the multiple interactions in which tetraspanins engage.

THE TETRASPANIN WEB: A DYNAMIC NETWORK OF INTERACTIONS

The origin of the tetraspanin web concept

The ability of tetraspanins to interact with one another and with a multitude of other transmembrane proteins was demonstrated in the mid-1990s. This unique property is best appreciated after labelling of surface proteins and immunoprecipitation of tetraspanins after lysis in appropriate detergents. Unlike many other immunoprecipitates, those of tetraspanins contain numerous surface-labelled proteins. Remarkably, for a particular cell type and under defined lysis conditions, the patterns of proteins co-immunoprecipitated with the different tetraspanins are identical [41–43]. Western blot experiments have confirmed that tetraspanins co-immunoprecipitate the same proteins with, however, different co-immunoprecipitation efficiencies, and have shown that all tetraspanins tested associate with one another [41–43]. On the basis of these data, it was proposed that tetraspanins organize a network of molecular interactions at the cell surface, thus coining the term 'tetraspanin web' [41]

The list of tetraspanin-associated proteins has grown over the years, especially through the proteomic analysis of tetraspanin-containing complexes [44–49]. Schematically, the proteins associating with tetraspanins can be classified (while not exclusively) into several major groups: integrins and other adhesion molecules [EpCAM (epithelial cell adhesion molecule), VCAM-1 (vascular cell adhesion molecule 1), ICAM-1 (intercellular cell adhesion molecular 1), etc.], proteins with Ig domains (CD9P-1/EWI-F, EWI-2, etc.) and ectoenzymes (including ectopeptidases and metalloproteinases), as well as intracellular signalling molecules such as heterotrimeric G-proteins, PI4K (phosphoinositide 4-kinase) and activated PKC (protein kinase C) [12,16,17,47,48].

Several levels of interaction in the tetraspanin web

The tetraspanin web can be viewed as one or several molecular jigsaw puzzles at the cell surface. Co-immunoprecipitation assays help to identify which pieces are involved, but provide no clue as to the number in each puzzle or the way in which each is assembled. This could be explained by the existence at the cell surface of a multitude of small and diverse complexes, or of bigger less varied structures. The size and the stoichiometry of the complexes remain unclear. Pre-clearing experiments have revealed that at least three tetraspanins could exist in one single complex [41,43], and gel filtration has indicated that most of the CD9- and CD81-containing complexes conserved after Brij 96 lysis are much smaller than 4×10^6 Da [50].

The comparison of tetraspanin immunoprecipitates after cell lysis using different detergents has helped to unravel the organization of the tetraspanin web. Indeed, certain detergents maintain the interactions of some tetraspanins with specific non-tetraspanin proteins, while dissociating tetraspanin–tetraspanin interactions. We define as 'partner proteins' those that remain associated with a given tetraspanin under conditions disrupting the tetraspanin–tetraspanin interactions. Several cross-linking experiments have indicated this as a level of direct interaction [45,46,51–54]. A list of established and potential partner proteins is provided in Table 1. The interaction of at least four tetraspanin–partner pairs (CD151–integrin $\alpha 3\beta 1$; CD81–CD19; UPs) occurs early in biosynthesis, probably before these molecules leave the endoplasmic reticulum [55–58].

Interactions occurring between partners of a given tetraspanin and other tetraspanins detectable only under lysis conditions preserving tetraspanin–tetraspanin interactions are most likely to be indirect and secondary to the tetraspanin–tetraspanin interactions [59]. Several pieces of evidence are in favour of this hypothesis. First, integrin $\alpha 3\beta 1$ transfected into a CD151-negative cell line only interacts with endogenously expressed tetraspanins when CD151 is also expressed [21]. Secondly, in CHO cells, a complex containing CD82 and CD9P-1 can only be observed when CD9, but not CD151, is co-transfected [21]. Thirdly, the expression of a CD151 mutant lacking all palmitoylation sites and defective for the interaction with other tetraspanins reduces the interaction of integrin $\alpha 3\beta 1$ with other tetraspanins [24]. Finally, the interaction of integrin $\alpha 3\beta 1$ with other tetraspanins is strongly reduced by the silencing of CD151 [60–62] and is not detectable in endothelial cells obtained from $CD151^{-/-}$ mice [63].

Table 1 Established and potential tetraspanin–partner pairs

FPRP, prostaglandin F2α receptor regulatory protein; PGRL, prostaglandin regulatory-like protein.

Tetraspanin	Partner	Confirmed by cross-linking	Reference	Comment
UP1a	UPII	Yes	Reviewed in [10]	The UP1b–UPIII pair served as a control
UP1b	UPIIIa	Yes	Reviewed in [10]	The UP1a–UPII pair served as a control
CD9	CD9P-1/EWI-F/FPRP	Yes	[46,52]	Specific digitonin-resistant complex. Partially resistant to Triton X-100 disruption
	EWI-2/PGRL	Yes	[45,53]	Specific digitonin-resistant complex
	EpCAM	Yes	[48]	Specific digitonin-resistant complex
	Pro-HB-EGF	Yes	[40]	Only CD9 can up-regulate its activity
	ICAM-1	No	[64,113]	FRET analysis suggests a proximity of ICAM-1 with CD9, but not CD151
	Claudin-1	Yes	[49]	CD81 and CD151 also associate with claudin-1. Cross-linking indicates proximity
CD81	CD19	No	[57,122,240,241]	Specific digitonin-resistant complex
	Integrin $\alpha4\beta1$	No	[59]	Specific digitonin-resistant complex
	CD9P-1/EWI-F/FPRP	Yes	[46,52]	Specific digitonin-resistant complex
	EWI-2/PGRL	Yes	[45,53,242]	Specific digitonin-resistant complex
	Claudin-1	No	[49,243]	CD9 and CD151 also associate with claudin-1. FRET indicates proximity
CD151	Integrin $\alpha3\beta1$	Yes	[51,59,103]	Specific digitonin- and Triton X-100-resistant complex
	Integrin $\alpha6\beta1$	Yes	[55,59]	Specific digitonin-resistant complex
	Integrin $\alpha6\beta4$	No	[108,160,161]	A direct association is suggested by the targeting of CD151 to hemidesmosomes by this integrin and the absence of staining of hemidesmosomes by a CD151 mAb probing the fraction of CD151 not associated with integrins.
	Integrin αIIb$\beta3$	+/−	[167]	Interaction observed in Triton X-100, but no other tetraspanin tested in parallel. Cross-linking experiments did not provide any information on the size of the complex
	VCAM-1	No	[64,113]	FRET analysis suggests proximity of VCAM-1 with CD151, but not CD9
	MT1-MMP	No	[157]	FRET analysis suggests proximity of MT1-MMP with CD151, but not CD9. Specific effect of CD151 silencing on MT1-MMP function
CD53	ADAM10	No		Specific digitonin-resistant complex (F. Le Naour and E. Rubinstein, unpublished work)
CD63	H^+/K^+-ATPase	No	[141]	Interaction observed in Triton X-100, but no other tetraspanin tested in parallel

The tetraspanin–partner pair model for the organization of the tetraspanin web is supported by the study of the urothelium, a stratified squamous epithelium covering much of the urinary tract. The urothelial apical surface is almost completely covered by hexagonally packed 16-nm UP particles, forming two-dimensional crystals also known as 'urothelial plaques'. Each 16-nm particle can be resolved into six dumbbell-shaped subunits, each consisting of an inner and an outer subdomain [19]. Highly purified urothelial plaques contain two tetraspanins, UPIa and UPIb, interacting respectively with the non-tetraspanin proteins UPII and UPIIIa. Available data suggest that the resulting UPIa–UPII and UPIb–UPIIIa heterodimers are associated with the inner and outer subdomain respectively [10].

The interaction of two tetraspanins in cells has recently been confirmed using FRET (fluorescence resonance energy transfer) [64], single-molecule tracking [65] and chemical cross-linking [66]. Tetraspanins can be cross-linked to one another not only through classical amine-reactive agents, but also more efficiently through membrane-permeant thiol-specific cross-linkers, provided that palmitoylation is prevented using the inhibitor 2-bromopalmitate [66]. These experiments yield bands consistent with the cross-linking of two or three CD9 or CD81 molecules. Bands containing the same tetraspanin are more readily detected than bands containing two different tetraspanins, leading to the suggestion that tetraspanin homodimers represent the building blocks for the tetraspanin web. However, alternative interpretations of this result cannot be excluded. Indeed, bands containing the same tetraspanin are expected to be more easily detected than bands containing two different tetraspanins, since, for an equivalent number of complexes, there are twice the number of sites for the relevant antibody. In addition, the distribution of tetraspanins in the membrane may not be equivalent, so that a better cross-linking of two copies of a given tetraspanin in certain cell lines could reflect the existence of areas enriched in that particular tetraspanin. Structural and biochemical analysis of the bladder tetraspanins UPIa and UPIb have provided no evidence for the existence of homodimers [10], whereas the retinal tetraspanin peripherin/RDS has been found to assemble as homotetrameric or heterotetrameric (with another tetraspanin, Rom-1) complexes [11,67].

Regions of tetraspanins involved in interactions with partners

One of the best-characterized tetraspanin–partner pairs is CD151–integrin $\alpha3\beta1$. Studies using chimaeras under conditions disrupting tetraspanin–tetraspanin interactions have demonstrated the requirement for the LED of CD151 for the interaction with integrin $\alpha3\beta1$ and that most of the constant domain could be replaced by that of other tetraspanins without compromising this interaction [51,55,56]. Point mutation analysis has more specifically highlighted the key role of three residues of the D subdomain of CD151, but which are, however, insufficient to mediate the interaction [55]. Other examples have underlined the complexity of the interactions. Indeed, a chimaera in which the CD9 LED is replaced by that of CD81 associates more strongly than CD9 with CD19 after Brij 97/EDTA lysis. However, the CD81 LED is not involved in the ability of CD81 to facilitate the exit of CD19 from the Golgi, which instead requires (in a CD9 backbone) the first TM only, suggesting the independent capacity of this domain to interact with CD19 [68]. Interestingly, the interaction of CD9 with EWI-2 is also mediated by two independent domains. One is located in the second half of CD9 LED (between the CCG motif and TM4) and the second is located in the TM2/TM3 region [53]. The interaction of CD9 or CD81 with CD9P-1 does not involve the TM2/TM3 region [52], but does depend fully on TM4 and/or the C-terminus (S. Charrin and E. Rubinstein, unpublished work). The analysis of urothelial plates by cryo-electron microscopy at 6 Å (1 Å = 0.1 nm) resolution revealed that the non-tetraspanin UPs interact with both the LED

and the TMs, most likely TM3 and/or TM4, of their tetraspanin partners [19]. This study did not reveal any direct interaction between tetraspanin UPs.

Technical issues related to the use of detergents in studying the tetraspanin web

Although a few primary complexes can be observed after lysis in Triton X-100, including CD151–integrin $\alpha3\beta1$ and CD9–CD9P-1, the majority of tetraspanin–partner pairs have been observed following cell lysis in digitonin (see Table 1). Some primary complexes may not, however, be detected in digitonin, as suggested by the use of other detergents [53]. This may be due to the fact that digitonin dissociates certain complexes, or alternatively due to the particular mode of action of this detergent. Indeed, digitonin forms a 1:1 complex with cholesterol and precipitates this lipid, as well as a fraction of tetraspanins, probably that tightly associated with cholesterol [69]. In addition, digitonin fails to precipitate tetraspanins if tetraspanin–tetraspanin interactions are disrupted [69], suggesting that it only precipitates those tetraspanins associated with one another, leaving in the supernatant tetraspanins either 'isolated' or associated with their partners. One could therefore argue that some tetraspanin–partner pairs are totally precipitated by this detergent owing to their association with other tetraspanins.

The results obtained with Brij 97 can vary greatly depending on the cell type or the buffer used. Brij 97-based lysis buffers supplemented with $CaCl_2$ and $MgCl_2$ invariably preserve tetraspanin–tetraspanin interactions. Whether the tetraspanin–tetraspanin interactions are conserved or not in the presence of EDTA depends on the cell line studied. One factor contributing to the higher resistance of tetraspanin–tetraspanin interactions after Brij 97/EDTA lysis is the expression of CD9 [53,70]. In other experiments, lysis in the presence of Brij 97 and $MgCl_2$ preserved the interaction between CD9 and CD81, but disrupted that with other tetraspanins [44,46]. This observed difference between EDTA- and bivalent cation-supplemented buffers may be due to a change in the properties of the lipid bilayer. Indeed, in membrane models, bivalent cations have been shown to increase the resistance of supported lipid bilayers to detergent solubilization [71], possibly as the result of a change in the membrane order. The adsorption of bivalent cations, especially Ca^{2+}, to the surface of membranes can increase their cohesion. Such lipid headgroup–ion interactions are highly specific and involve the ions, the polar part of the lipid and the hydration layer [72]. In contrast, EDTA can induce fluidization of the membrane [73].

Among the different detergents that preserve tetraspanin–tetraspanin interactions, we prefer to use Brij 97 over CHAPS, Brij 58 or Brij 98. The latter three detergents yield a higher background compared with Brij 97 and a reduced ability to solubilize tetraspanins as judged by the presence of tetraspanins in the sedimentable fraction after centrifugation of the lysates at 10000 ***g***. Several proteins, for example integrins $\alpha2\beta1$ and $\alpha5\beta1$, are co-immunoprecipitated with tetraspanins after lysis with the milder detergents (Brij 98, Brij 58 or CHAPS), but not in more stringent detergents such as Brij 97, digitonin or Triton X-100 (M. Billard and E. Rubinstein, unpublished work). In addition, the interactions revealed with the milder detergents have not been confirmed using alternative approaches such as chemical cross-linking. Therefore, considering the poor solubilizing ability of these mild detergents, the physiological relevance of the interactions visualized uniquely using these detergents remains uncertain.

Dynamics of the tetraspanin web

The dynamics of the tetraspanin web has recently been addressed by studying single fluorescent CD9 molecules using TIRF (total internal reflection fluorescence) microscopy, as well as FRAP (fluorescence recovery after photobleaching) and FCS (fluorescence correlation spectroscopy). The majority of the CD9 molecules display a Brownian diffusion mode, at least during part of their trajectory, underlining the highly dynamic behaviour of CD9. The diffusion of CD9 and CD151 ($\sim0.2\ \mu m^2/s$) is similar to that of other membrane proteins [64,65].

FRET analysis indicated an interaction of tetraspanins outside of tetraspanin-enriched areas [64]. The tracking of single fluorescent CD9 molecules revealed the frequent and transient co-diffusion of two CD9 molecules undergoing Brownian trajectories [65], suggesting that tetraspanin–tetraspanin interactions occur transiently and in succession (Figure 3). No abrupt change in CD9 velocity was observed when the molecules started to co-diffuse. As one would expect the diffusion of transmembrane proteins in liquid membranes to decrease with increasing size of the diffusing protein [74], this observation seems to exclude the interaction of two isolated CD9 molecules. We therefore suggest that CD9 diffuses in the membrane, embedded in a small membrane cluster able to accommodate diverse proteins (in particular several tetraspanins and their partners) and lipids, and that these clusters either co-diffuse or exchange tetraspanins (and their partners). The hypothesis that several tetraspanins are present in small clusters is supported by the fact that anti-tetraspanin mAbs induce the regrouping of several tetraspanins in membrane patches, indicating the association of more than two tetraspanins at the same time in the diffusing fraction [75].

In about a third of the trajectories, CD9 molecules are transiently confined in areas enriched in CD9, CD81 and CD9P-1 [65], in which tetraspanin–tetraspanin interactions may be amplified (Figure 3). In this regard, in experiments where CD9 was tracked using intact mAb instead of Fab fragments, the CD9 molecules entering the platform did not exit, presumably because of the mAb-mediated stabilization of interactions with other CD9 molecules [65].

How are these tetraspanin-enriched areas assembled and are they induced by physiological stimuli? Their maintenance does not depend on cholesterol or the actin cytoskeleton [65]. One possibility is that one or several molecules associated with the tetraspanin web are immobilized by a ligand, leading to the subsequent confinement of diverse tetraspanins. In this regard, Barreiro et al. [64] demonstrated that engagement of endothelial VCAM-1 or ICAM-1 by cells expressing their ligands induces the formation of a docking structure containing these two adhesion receptors and enriched in tetraspanins. Interestingly, the diffusion of CD9 in this structure is diminished compared with its diffusion in the plasma membrane, suggesting that this docking structure may resemble the tetraspanin-enriched areas observed in cancer cells by TIRF microscopy [65].

The role of lipids in building the web

A number of membrane components are poorly solubilized in detergents and partition into the low-density fractions of sucrose gradients. The raft concept has provided a possible explanation for these DRMs (detergent-resistant membranes), the idea being that lipids with long saturated chains interact tightly with cholesterol and are thereby subjected to phase separation [76]. Although such phase separation has been observed in model membranes, its existence in biological membranes is still a matter of debate. Considering the limits of the DRM approach, the association of membrane components with DRMs does not necessarily reflect

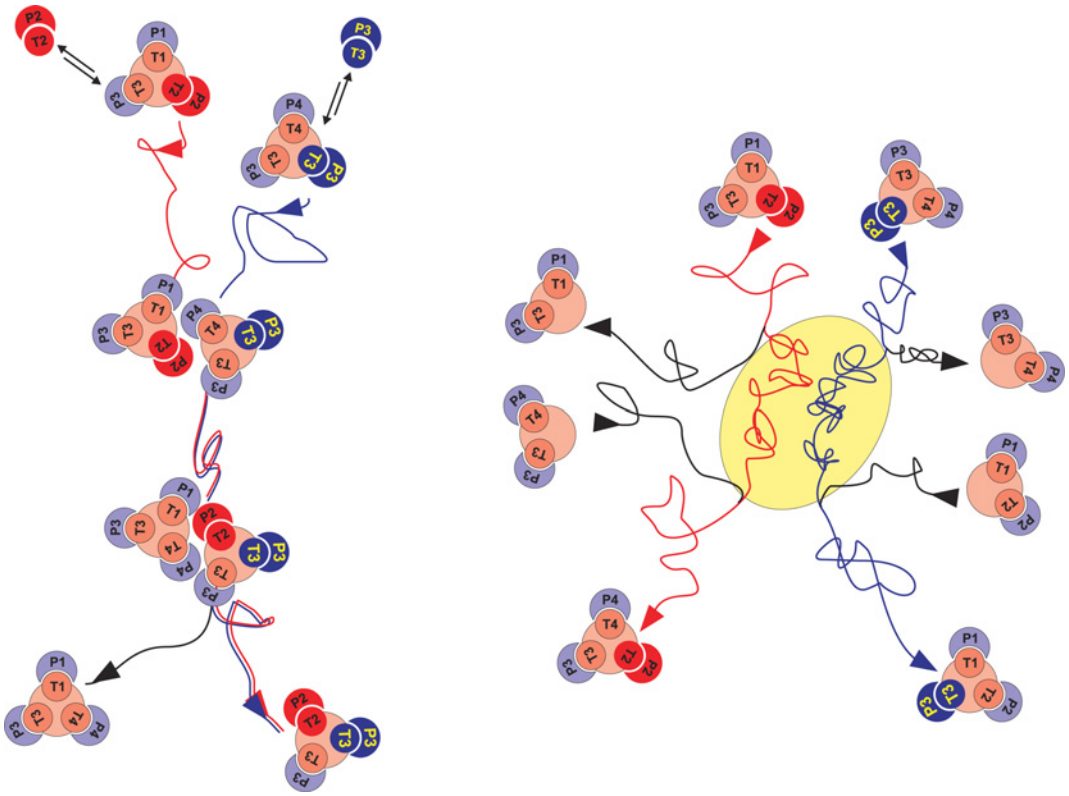

Figure 3 A dynamic view of the tetraspanin web

This model is based on biochemical analysis of the tetraspanin web and the recent analysis of the dynamics of CD9 and CD151. For clarity, two tetraspanin–partner pairs are labelled in red and blue, and the traces correspond to their movement. Left: basal level of interactions. Small clusters of tetraspanins (T1, T2, etc.), each specifically associated with a molecular partner (P1, P2, etc.), would patrol in the plasma membrane, frequently interacting with other clusters and exchanging some of their constituents, contributing to the diversity of interactions within the tetraspanin web. An animated version of this Figure can be seen at http://www.BiochemJ.org/bj/420/0133/bj4200133add.htm. Right: upon particular stimulations, which remain largely to be defined, some tetraspanins become confined within discrete areas of the plasma membrane where more stable interactions take place. The model shown here is based on the transfer of one tetraspanin to the tetraspanin-enriched areas, but other models are possible, such as the gathering of several clusters.

their localization in rafts, but it may give information on the membrane environment [77].

Similarly to raft resident proteins, tetraspanins partition into low-density fractions of sucrose gradients, indicating their localization in DRM. This partitioning is best seen in detergents that preserve tetraspanin–tetraspanin interactions (CHAPS, Brij 96/97, Brij 99) [21,24,50,78], leading to the hypothesis that tetraspanin complexes form specialized microdomains named tetraspanin-enriched microdomains [24,50]. It should be noted that tetraspanins can interact with one another or non-partner associated proteins outside of the DRM [50,70], indicating that their association is not due to resistance to solubilization.

Several differences exist between rafts or GPI (glycosylphosphatidylinositol)-microdomains and the tetraspanin web. The partitioning of tetraspanins into low-density fractions is minimal after cell lysis in Triton X-100, a condition which preserves the GPI-microdomains and disrupts tetraspanin–tetraspanin interactions [21,50]. In addition, cell lysis in a Brij detergent at 37 °C disrupts rafts, but preserves the interaction of tetraspanins and their ability to float in the low-density fraction [21,50]. Furthermore, compared with rafts, the tetraspanin complexes are more difficult to disrupt with cholesterol-depleting agents ([50], see below). Several studies report the lack of interaction of tetraspanins with raft-resident molecules such as GPI-anchored molecules [50,53,79] or caveolin [24,50]. Concordantly, proteomic analysis of rafts has identified no tetraspanins, and that of CD9 complexes yielded no caveolin or GPI-anchored proteins [47,48,80]. Finally, single-molecule tracking of CD9 and the GPI-anchored molecule CD55 has highlighted their different behaviours [65]. Altogether, these data provide evidence for the distinct nature of the tetraspanin web and ‘classical’ rafts.

The interaction of tetraspanins with certain lipids is tight enough to be visualized using radiolabelled photoactivatable compounds. In this way, CD9 was labelled with a photoactivatable G_{M3} derivative under conditions where no labelling of several integrin subunits could be detected [81]. Similarly, several tetraspanins, including CD9, CD81 and CD82, were labelled with a cholesterol analogue, whereas no labelling of MHC class I heavy chain could be detected [69]. The interaction of tetraspanins with cholesterol is supported further by the finding that digitonin, a well-known cholesterol-precipitating reagent, precipitates these molecules [69]. How tetraspanins interact with cholesterol is unknown. The recent determination of the crystal structure of the G-protein-coupled human β_2-adrenergic receptor revealed the role of two cholesterol molecules that interact with palmitic acid molecules carried by the receptor for receptor interaction [82]. We therefore speculate that tetraspanin palmitate moieties mediate this interaction with cholesterol. In this regard, a palmitoylation-free mutant CD9 molecule was not precipitated by digitonin under particular conditions [69]. Alternatively, several asymmetrically clustered membrane-exposed aromatic residues revealed following the modelling of tetraspanins, have

been suggested to mediate the interaction with cholesterol [30].

Lipids may play an important role in the interaction of tetraspanins with one another and therefore in the building of the tetraspanin web. Indeed, inhibition of the glycosphingolipid biosynthetic pathway reduces the interaction of CD82 with CD9 and CD151 in mammary epithelial cells. In addition, ectopic expression of sialidase Neu3, a cell-surface enzyme that removes sialic acid from gangliosides G_{D1a} and G_{M3} at the plasma membrane, reduces the interaction of CD82 with CD151 [83]. However, manipulating G_{M3} levels does not affect the interaction of CD82 with other tetraspanins, thus underlining the specific involvement of the ganglioside G_{D1}. Importantly, modulation of membrane ganglioside composition does not affect the interaction of other tetraspanins with one another. Therefore G_{D1} specifically controls the interaction of CD82 with other tetraspanins [83]. In addition, expression of CD82 has been associated with an increased expression of G_{D1a} and G_{M1} [84].

The role of cholesterol in the assembly of the tetraspanin web is still under debate. Treatment of intact cells with MβCD (methyl-β-cyclodextrin), a drug which does not incorporate into the membrane, but extracts membrane cholesterol by including it in a central non-polar cavity, did not reduce tetraspanin–tetraspanin interactions as determined by co-immunoprecipitation [50,69]. By contrast, cell lysis in the presence of MβCD or saponin (a cholesterol-sequestering reagent) significantly disrupts these interactions [69]. To explain this discrepancy, we propose that the cholesterol found in the inner leaflet partly mediates the interaction of tetraspanins with one another, possibly by interacting with palmitate moieties, and that this pool of cholesterol is not readily extractable by MβCD.

The pool of cholesterol that is extractable by MβCD does play an important role in the behaviour and function of tetraspanins. Indeed, cholesterol depletion by MβCD modifies the behaviour of CD9 molecules analysed at the single-molecule level [65] and decreases the binding of an anti-CD81 mAb that recognizes CD81 only when it is associated with other tetraspanins [85]. In addition, treatment of live cells with MβCD was shown to inhibit the CD81-dependent infection of liver cells by malaria sporozoites [85], the tetraspanin ligation-induced tyrosine phosphorylation in lymphoid B-cells [69], and the CD82-dependent functional effects in Jurkat T-cells [78].

Several reports have shown that mutation of the intracellular membrane proximal cysteine residues, the site of attachment of palmitate moieties, partially reduces the interaction with other tetraspanins [21–24,70]. Concordantly, overexpression of DHHC2, a protein acetyltransferase mediating palmitoylation of tetraspanins, increases the interaction between CD9 and CD151 [86]. In one study, mutation of all palmitoylation sites of CD9 barely affected the interaction with other tetraspanins as determined after lysis with Brij 97 in the presence of bivalent cations. However, the interactions are disrupted when bivalent cations are replaced by EDTA [70]. This demonstrates the dispensability of the palmitate moieties for interactions between tetraspanins, and suggests that, rather than initiating these interactions, they may act to stabilize them. The palmitate residues may also modify the dynamics of interaction between tetraspanins [65].

THE INNER WORLD OF THE TETRASPANIN WEB

Role of tetraspanin cytoplasmic domains

Except for the N-terminal tail of Tspan-10 and the C-terminal tails of Tspan-10, Tspan-12/NET-2, Tspan-15/NET-7, Tspan-32, Tspan-33, peripherin/RDS and Rom-1, the cytosolic extremities of tetraspanins are typically less than 20 residues [12]. Despite being more conserved, no function has so far been attributed to the N-terminal cytosolic domain. In contrast, the importance of the C-terminal cytosolic domain is coming to the fore.

Several tetraspanins contain a tyrosine-based internalization motif YXXΦ (where Φ is a hydrophobic amino acid, and X is any amino acid) [87]. Mutation of this site in CD151 prevents its accumulation in intracellular vesicles and reduces endocytosis. The location of the YXXΦ motif in CD63 at the end of the C-terminal domain and preceded by a glycine residue, is unique among tetraspanins and critical to the sorting of CD63 into late endosomes/lysosomes. It also confers on CD63 the ability to interact with the μ subunits of several AP (adaptor protein) complexes, components of protein coats that participate in cargo selection and vesicle formation in the endocytic and late secretory pathways [87,88].

Latysheva et al. [89] reasoned that tetraspanins may interact with other proteins present in exosomes. Syntenin-1 was shown to interact with several tetraspanins under conditions preserving the interaction of tetraspanins with integrin $\alpha3\beta1$ (0.8 % Brij 98, 0.2 % Triton X-100), but with CD63 only using higher concentrations of Triton X-100. This suggested that, of the tetraspanins tested, only CD63 interacted directly with syntenin-1. In accordance with these findings, a C-terminal peptide from CD63, but not CD151 or CD9, was found to interact *in vitro* with recombinant syntenin-1. Syntenin-1 is a protein with a PDZ domain that recognizes the last amino acids at the C-terminus of target proteins [90]. A PDZ-binding sequence found on CD63 was found to be responsible for the interaction with syntenin-1 [89]. Overexpression of syntenin-1 decreases the rate of constitutive internalization of CD63. However, neither the overexpression nor the silencing of syntenin-1 affects the steady-state distribution of CD63 in HeLa cells. Because the PDZ-binding motif of CD63 overlaps the YXXΦ binding motif, it was proposed that syntenin-1 could compete with the μ-adaptor subunits for binding to CD63. Other tetraspanins such as CD9, CD81 and CD151 have a PDZ-binding sequence which may be involved in the binding of other PDZ proteins. In this regard, a peptide corresponding to the C-terminus of CD81 was shown to interact with several PDZ domains, and co-immunoprecipitation revealed an interaction between CD81 and the PDZ domain proteins EBP50 [ERM (ezrin/radixin/moesin)-binding phosphoprotein 50] and SAP97 (synapse-associated protein 97) [91]. Interestingly, EBP50 strongly associates with ezrin (see below). Whether PDZ proteins contribute to the interaction of tetraspanins with other transmembrane proteins remains to be determined.

Tetraspanins, ERM proteins and the actin cytoskeleton

Anti-CD82 mAb (alone or in combination with anti-CD3 mAb) immobilized on plastic induce major morphological changes in Jurkat cells, relating to the formation of long membrane extensions and actin cytoskeleton rearrangements [92]. Several lines of evidence indicate that these cytoskeleton rearrangements are important for the ability of anti-CD82 mAb to co-stimulate T-cells. First, inhibition of cytoskeleton rearrangements by cytochalasin blocks the ability of anti-CD82 mAb to potentiate the tyrosine phosphorylation induced by suboptimal doses of anti-CD3 mAb [92]. Secondly, inhibition of Rho GTPases inhibits both morphological changes and the enhancement of tyrosine phosphorylation [93]. Immobilized anti-CD81 mAb also induces morphological changes of both primary T-cells and NK (natural killer) cells. Anti-CD81 (and anti-CD82) mAb

was shown to inhibit NK cell activation by various stimuli, an effect which could be reversed by blocking actin polymerization [94,95]. Importantly, blocking actin cytoskeleton rearrangement is sufficient to potentiate the activation of NK cells by anti-CD16 mAb [95]. In contrast, the same treatment inhibits the activation of T-cells by immobilized anti-CD3 mAb. Thus blockers of actin cytoskeleton rearrangement and immobilized anti-CD81 mAb produce opposite effects in T- and NK cells. Interestingly, a parallel situation was noted in mast cells where anti-CD81 mAb blocked activation, whereas actin blockers increased it [96,97]. The authors suggested that, through their effect on the cytoskeleton, tetraspanins may modulate activation thresholds, leading to opposite outcomes in T- and NK cells [95].

The interaction of tetraspanins with the cytoskeleton may occur through proteins of the ERM family. These proteins interact with actin through their C-terminal domain, and with the cytoplasmic domain of several adhesion molecules through their N-terminal domain [98]. Tetraspanins CD9 and CD81, but not CD151, have been shown to co-immunoprecipitate ERM proteins. The C-terminus of CD81 pulled down both ezrin and moesin, suggesting a direct interaction [99]. However, both ERM proteins were also pulled down by the cytoplasmic domain of CD9P-1 and EWI-2, suggesting that an indirect interaction between tetraspanins and ERM proteins may also exist. In polarized leucocytes, CD9 and CD81, as well as EWI-2, are found concentrated with ERM proteins at a membrane protrusion found at the rear part of the cell body called the uropod. The relevance of this interaction is not yet clear, because the ERM-protein-dependent localization of EWI-2 to the uropod does not require its cytoplasmic domain. Silencing of EWI-2 was found to slightly increase the polarization of a lymphoid cell line, its ability to migrate, as well as ERM protein phosphorylation [99].

Few signalling molecules have been identified in the tetraspanin web

Compared with the wealth of cell-surface molecules associating with tetraspanins, only a few cytoplasmic molecules have been shown to interact with tetraspanins. Unless specified above, it is not known whether any of these interactions are direct. The only intracellular proteins associating with CD9 [47,48], as identified by recent proteomic analysis, are syntaxins 3 and 4A (two proteins involved in vesicle fusion), PKCβ and a subset of heterotrimeric G-proteins, corroborating the results of Little et al. [44]. The functional relationship between tetraspanins and heterotrimeric G-proteins remains unclear. However, as the same fraction of the orphan heterotrimeric GPCR (G-protein-coupled receptor) GPR56 associating with $G_{\alpha q/11}$ also associates with CD81, and as the expression of CD81 increases the interaction between GPR56 and $G_{\alpha q/11}$, Little et al. [44] suggested the potential role of CD81 as a heterotrimeric GPCR scaffolding protein [44]. Scaffolding proteins are believed to contribute to GPCR signalling specificity by engaging additional signalling pathways or localizing GPCR signalling events to specific subcellular sites [100].

Several tetraspanins (but not all) have been shown to associate with the lipid kinase PI4K [101] and PKCα and PKCβ2 [47,102]. In addition, only those integrins associating with tetraspanins interact with PI4K and PKCβ2 [102,103]. Thus tetraspanins may link activated PKC and PI4K to the subset of integrins with which they interact. However, whether these kinases interact directly with one or several tetraspanins is unclear. The interaction of CD81 and CD151 with PKC could be stabilized through cross-linking reagents; however, the minimum size of complexes containing both tetraspanins and PKC is unknown [102,103]. In addition, the use of chimaeric molecules consisting of CD9 and Tspan-7 (TALLA-1), which does not associate with PKC, has yielded no conclusive results on the role of CD9 cytoplasmic domains in mediating this association.

Finally, the non-palmitoylated form of CD81 was shown to associate with the serine/threonine-binding signalling protein, 14-3-3. Both the palmitoylation of CD81 and association with 14-3-3 were modulated by oxidative stress [104].

REGULATION OF TETRASPANIN-ASSOCIATED PROTEINS THROUGH THE TETRASPANIN WEB

Potential role of the tetraspanin web and the difficulty in assigning tetraspanin-specific functions

What is the function of the tetraspanin web? We suggest that tetraspanins, through the organization of the tetraspanin web, contribute to the lateral segregation of the proteins with which they associate. More specifically, on the basis of the biochemical dissection of the tetraspanin web and the analysis of the dynamics of some of its constituents, we propose that one major role for tetraspanins is in the assembly of specialized structures. To achieve this function, tetraspanins, associated with their partners, would patrol the membrane and generate a succession of transient interactions. Since the engagement of one tetraspanin partner through its ligand would be accompanied by the co-recruitment of other tetraspanin partners, these transient interactions would therefore facilitate the assembly of more complex structures (Figure 3). In this regard, integrins have been shown to associate with other tetraspanin-associated molecules such as MHC molecules, CD9P-1 or EWI-2 under conditions preserving tetraspanin–tetraspanin interactions [21,41,60,105]. This model is also coherent with the finding that engagement of endothelial VCAM-1 or ICAM-1 by cells expressing either one of their ligands induces the formation of a docking structure containing both adhesion receptors and tetraspanins [64]. In particular, tetraspanins may favour the association of several copies of a given associated protein, as suggested by the CD9-dependent association of several MHC class II molecules [106]. Such microclustering of certain proteins could have important consequences on their function. The tetraspanin web may also serve as a regulated adaptor platform between protein partners and intracellular signalling molecules such as PI4K or PKC [101,102]. Finally, one cannot exclude the possibility that the interaction with tetraspanins may restrict other interactions of some cell-surface proteins.

A major difficulty in the study of tetraspanins is determining functions that are specific to a given tetraspanin and how they relate to specific tetraspanin-associated proteins. Considering the wealth of molecular interactions involving tetraspanins, ensuring that the observed effects are linked to the specific function of the tetraspanin initially targeted and not secondary to a modification of the tetraspanin web is a challenge. This is especially true for experiments using mAb against tetraspanins. These mAbs similarly engage the tetraspanin web, with mAbs against different tetraspanins often concentrating several tetraspanins in discrete membrane areas or producing similar effects. Indeed, earlier studies have reported that, when tested in parallel, different anti-tetraspanin mAbs all induced co-stimulation of T-cells, cell aggregation, and modified cell migration (for a review, see [12]). However, there are some examples where mAbs have helped to unravel tetraspanin-specific functions. For example, it was shown that an anti-CD151 mAb, but not an anti-CD9 mAb, blocked the formation of cellular cables [107] (see below). The use of particular mAbs that dissociate one tetraspanin from

its partners would also be useful [108,109]. One might also expect the recombinant tetraspanin LED to be more useful in terms of determining specific functions. However, different tetraspanin LEDs also seem to produce the same range of effects, including inhibition of sperm-fusion and inhibition of HIV infection [110,111]. In addition, recombinant CD9 LED was recently shown to reduce the diffusion of both CD9 and CD151 [64].

Will RNA silencing and gene knockout prove more powerful in determining tetraspanin-specific functions? In a recent study, CD151 silencing reduced invasion through Matrigel, whereas CD9 silencing had a stimulatory effect [62]. However, in other studies, the silencing of CD9 or CD151 in melanocytes or endothelial cells produced similar functional effects, and T-cells from several tetraspanin-null mice are hyperproliferative, indicating that gene silencing may affect functions common to different tetraspanins [17,112]. These common effects may be due to a modification of the organization of the tetraspanin web or to a change in expression of another tetraspanin, as suggested by the fact that CD151$^{-/-}$ keratinocytes show reduced CD9 expression. They may also be due to the down-regulation of some associated proteins as shown by the decreased expression of ICAM-1 and VCAM-1 upon CD9 and CD151 silencing [113].

Similarly, transfection studies should also be interpreted cautiously. For example, CD82 expression in several cell lines has been shown to modify signalling events downstream of integrins and EGFR (epidermal growth factor receptor). However, CD82 expression regulates the level of some gangliosides which themselves contribute to the interaction of CD82 with other tetraspanins. In addition, EWI-2 overexpression in lymphoid T-cells impaired spreading and ruffling on VCAM-1, an integrin $\alpha4\beta1$ ligand [105], but in other cells, it changed the organization of the tetraspanin web as shown by altered CD81 localization and modified binding of an anti-CD9 mAb [114,115].

While keeping these limitations in mind, one can nevertheless distinguish four major themes emerging from studies addressing the potential functional relationship between tetraspanins and their associated molecules: (i) modulation of ligand binding; (ii) modulation of signalling; (iii) modulation of trafficking; and (iv) regulation of proteolytic activities.

Modulation of ligand binding and adhesion strengthening

CD9 is associated with the membrane precursor of HB-EGF (proHB-EGF), a member of the EGFR ligand family, which serves as a receptor for diphtheria toxin. Expression of CD9, but not other tetraspanins, together with HB-EGF increases the sensitivity of cells to this toxin [40]. In addition, CD9 increases the juxtacrine activity of proHB-EGF [116]. Expression of CD9 in cells expressing proHB-EGF induces a 20-fold increase in the number of sites for diphtheria toxin without changing the affinity or number of proHB-EGF molecules present at the cell surface. It is therefore likely that CD9 modifies the conformation of proHB-EGF, thus uncovering new binding sites for the toxin.

Because the interaction of CD151 with the integrin $\alpha3\beta1$ is extremely stable, these molecules are often co-purified. The complex can, however, be dissociated using a specific anti-CD151 mAb (8C3). Liposomes reconstituted with the integrin $\alpha3\beta1$ after dissociation of CD151 show reduced binding to laminin 10/11 (an integrin $\alpha3\beta1$ ligand) compared with those reconstituted with the integrin $\alpha3\beta1$–CD151 complex [109]. This mAb also slightly inhibits the adhesion of cells to laminin 10/11, as does a 90% silencing of CD151. Treatment with the mAb also decreases the binding of an anti-($\beta1$ integrin) mAb reported to preferentially recognize 'activated' $\beta1$ integrins, leading to the suggestion that CD151 stabilizes an active integrin $\alpha3\beta1$ conformation.

Integrin-mediated adhesion is a dynamic multi-step process involving the binding of integrin receptors to extracellular ligands followed by the highly regulated post-binding events comprising receptor clustering and interactions with cytoskeletal structural and signalling elements that may contribute to adhesion strengthening [117]. To investigate a possible influence of CD151 on the strength of integrin adhesion, Lammerding et al. [118] allowed magnetic beads coated with laminin-1 (an integrin $\alpha6\beta1$ ligand) or fibronectin (an integrin $\alpha5\beta1$ ligand) to attach to cells and, using a magnetic trap, measured the force necessary to detach these beads. They showed that beads coated with laminin had normal initial binding, but were more easily detached from cells expressing a CD151 chimaera in which TM4 and the cytosolic C-terminal region had been replaced by that of Tspan-4 (NAG-2). This mutant did not affect the detachment of beads coated with fibronectin, demonstrating its specific effect on integrin $\alpha6\beta1$ which associates directly with CD151 [118]. Therefore CD151 has emerged as a regulator of integrin adhesion strengthening. This may explain why cells expressing the CD151 mutant show defects in spreading and cellular cable formation on Matrigel, a process also blocked by anti-integrin and anti-CD151 (but not anti-CD9) mAbs [107]. It may also explain why, in another study, CD151-silenced cells showed normal adhesion to laminin, but were more easily detached when subjected to centrifugation [119].

Tetraspanins can modulate signalling downstream of associated proteins

Engagement of tetraspanins by specific mAbs has long been known to trigger, in certain cells, signal transduction events, including tyrosine phosphorylation (for a review, see [12]) and activation of MAPKs (mitogen-activated protein kinases) [120,121]. How these cell-type-dependent signalling events are initiated is unclear. The most straightforward explanation is that engagement of tetraspanins induces their clustering and that of associated molecules, some of which would have signalling activities. For example, in lymphoid B-cells, tetraspanins associate with CD19, a molecule able to recruit several intracellular effectors, including Vav. Antibody-ligation of tetraspanins induces the phosphorylation of CD19 and Vav in the same way as the ligation of CD19 [69,122]. In the present review, we only review data showing that tetraspanins modulate the signalling ability of associated proteins. At this stage, however, it remains unclear as to whether tetraspanins modulate this signalling activity via coupling to specific signalling pathways.

Modulation of growth factor receptor signalling

Changes in tetraspanin expression have been shown to modulate the activity of both the EGFR and the HGF (hepatocyte growth factor) receptor c-Met. Investigations into the mechanisms involved during CD82 modulation of EGFR activity have provided a striking example of how tetraspanins can indirectly alter the function of other molecules.

Co-immunoprecipitation of CD82 with the EGFR or c-Met has been observed after cell lysis with mild detergents such as Brij 98. These interactions, like that of CD82 with other tetraspanins, are regulated by the expression levels of gangliosides [83,123–125]. CD82 was shown to negatively regulate the activation of the EGFR or c-Met and/or downstream signalling to some extent [123–126]. For example, initial phosphorylation of the EGFR

and Shc (a downstream adaptor) was shown to be normal, but slightly less stable, in cells transfected with CD82, possibly as a consequence of a more rapid EGFR internalization [123]. How CD82 modulates EGFR internalization and signalling is unlikely to be straightforward. Indeed the transfection of CD82 leads to an increase in the level of G_{M1} and G_{D1a}, gangliosides known to regulate the activity of several growth factor receptors, including EGFR and c-Met [127,128]. Some gangliosides have a positive effect on growth factor receptor activation, whereas others have a negative impact. In this way, the inhibition of ganglioside synthesis either increases or inhibits the activation of EGFR or c-Met, which may reflect different ganglioside composition. Importantly, whether stimulatory or inhibitory, the effect of inhibiting ganglioside synthesis on EGFR or c-Met activation was found to depend on the presence of CD82 [83,124,125].

Tetraspanin expression modulates integrin signalling

Several investigators have examined whether modifying the expression of tetraspanins or targeting tetraspanins with mAb could modify signalling downstream of integrins. Two major pathways are regularly, although not systematically, modified upon variation of tetraspanin expression levels: the PI3K (phosphoinositide 3-kinase)/Akt pathway and the FAK (focal adhesion kinase)/Src/$p130^{Cas}$ (Crk-associated substrate) pathway.

Takeda et al. [63] reported the reduced phosphorylation of Akt and its downstream target eNOS (endothelial nitric oxide synthase), both playing an important role in angiogenesis, in lung endothelial cells of $CD151^{-/-}$ mice upon plating on to Matrigel. Conversely, overexpression of CD151 in HUVECs (human umbilical vein endothelial cells) was shown to increase the level of Akt phosphorylation, in relation to an increase in PI3K expression [129]. In another study, however, cells expressing a palmitoylation-deficient mutant of CD151 had a higher level of phosphorylation of Akt than cells expressing wild-type CD151 [24].

Manipulating CD151 expression does not lead to a modification of Akt in all cell types. For example, silencing CD151 in mammary carcinoma cells does not modify the level of Akt phosphorylation upon plating the cells on laminin-1 [62]; however, it does impair the phosphorylation of FAK. This is consistent with the results of Yamada et al. [61], who observed a decrease in the phosphorylation of Src, FAK, $p130^{Cas}$ and paxillin upon plating of CD151-silenced A549 cells on LN-511 (laminin 10, an integrin $\alpha3\beta1$ ligand). Importantly, the same effect was observed in cells treated with an anti-CD151 mAb that dissociates CD151 from the integrin $\alpha3\beta1$, indicating a mechanism whereby CD151 modulates integrin signalling directly.

Other tetraspanins have been shown to modulate integrin signalling. Indeed, CD82 expression in a prostate carcinoma cell line diminished Src activation upon plating on various integrin substrates such as collagen, laminin-1 and fibronectin. It also impaired the phosphorylation of FAK at the Src-specific phosphorylation site, although not at the FAK autophosphorylation site, as well as that of its substrate $p130^{Cas}$, but did not alter ERK or Akt signalling [130]. This effect of CD82 on the Src/FAK/$p130^{Cas}$ pathway is not necessarily direct. In another study using a different prostate cancer cell line, the expression of CD82 (but not that of a palmitoylation-defective mutant of CD82) up-regulated the expression of FAK and Lyn at both the RNA and protein levels. In contrast, the level of $p130^{Cas}$ protein (though not RNA) was diminished, resulting in the formation of less $p130^{Cas}$–CrkII complex in cells expressing CD82 [23,131]. Overexpression of $p130^{Cas}$ reversed the CD82-mediated inhibition of cell motility. It is as yet unknown whether gangliosides contribute to this CD82-mediated effect on integrin signalling.

In other examples, lower levels of FAK phosphorylation have been found in $CD9^{-/-}$ vascular smooth muscle cells under conditions where they migrate [132]. In addition, transfection of CD9 has been associated with either an increase or a decrease in adhesion-dependent phosphorylation of Akt [133,134]. Finally, an anti-CD63 mAb was found to reduce the activation of mast cells plated on integrin substrates via the inhibition of a Gab2/PI3K, Akt and PKCδ pathway [135].

CD81 may modulate CD19-mediated B-cell signalling

CD19, a transmembrane protein that serves as a signalling/adaptor protein, is a major partner of CD81 in lymphoid B-cells. CD19 also associates directly with CD21, the receptor for the C3d component of complement, and provides a physical link between CD21 and CD81. When co-ligated to the BCR (B-cell receptor) through the binding of complement-tagged antigens, the CD19–CD21 complex functions to enhance BCR signalling, thus lowering the threshold for B-cell activation [136].

In untreated B-cells, the different components of the BCR (Igμ, Igα, Igβ) and the CD19–CD21–CD81 complex are fully solubilized by Triton X-100 at 4 °C and therefore partition in high-density fractions of sucrose gradients. Engagement of the BCR at 4 °C by mAb or multivalent antigens causes a fraction of BCR components, as well as proteins phosphorylated on tyrosine residues, to become resistant to detergent solubilization and therefore float in sucrose gradients. Incubation of the cells at 37 °C between the antibody-ligation and -lysis steps restores the solubilization of these proteins [137].

Antibody cross-linking of the BCR with CD19 or CD21 at 4 °C results in extensive partitioning of not only BCR subunits, but also CD19, CD21 and CD81 into the low-density fraction of sucrose gradients, with all molecules remaining partially insoluble to Triton X-100 even after incubation at 37 °C. This effect was not observed in B-cells from $CD81^{-/-}$ mice, indicating that CD81 plays a key role in rendering these molecules resistant to detergent solubilization. In these experiments, the level of global protein phosphorylation was lower in $CD81^{-/-}$ cells than in $CD81^{+/+}$ cells, with no phosphorylated proteins observed in the low-density fraction. In addition, no detectable phosphorylation of the BCR subunit Igα or CD19 was observed [138]. To what extent these two phenomena are due to a lower CD19 expression in $CD81^{-/-}$ cells, and whether the difference between $CD81^{+/+}$ and $CD81^{-/-}$ cells is amplified by performing the experiments at 4 °C, is unknown.

Tetraspanins sometimes regulate the trafficking of associated proteins

Several studies have addressed the question of whether tetraspanins regulate the traffic of some of the proteins they associate with. A detailed review of this aspect of tetraspanins has been published previously [87].

Regulation of biosynthetic transport and maturation

The cell-surface expression of CD19 on mature lymphoid B-cells is reduced by approx. 50 % in all strains of $CD81^{-/-}$ mice, with even lower expression levels observed in immature B-cells [17,57]. CD81 was shown to facilitate egress from the endoplasmic reticulum to the Golgi, and also contribute to the normal glycosylation of CD19 [68]. In addition, in transfected cells, UPII and UPIII only reach the plasma membrane by forming

heterodimeric complexes with UPIa and UPIb respectively [58]. Furthermore, expression of CD81 in U937 cells ectopically expressing hEWI-2 increases the surface expression of this partner [114].

Other tetraspanin partners do not require their paired tetraspanin to reach the cell surface. For example, integrin $\alpha3\beta1$ transfected into Daudi cells that do not express CD151 was found strongly expressed at the cell surface and showed no change following subsequent transfection of CD151 [21]. Concordantly, silencing of CD151 in A431 carcinoma cells does not reduce cell-surface expression of integrin $\alpha3\beta1$ [60]; neither does the knockdown of CD81 and CD9 alter the expression level of CD9P-1 in K562 cells (T. Abache and E. Rubinstein, unpublished work). Finally, the level of expression of integrins $\alpha3\beta1$ and $\alpha6\beta4$ is normal in isolated CD151$^{-/-}$ keratinocytes [139], and no changes in the distribution of CD9P1 expression in various organs of CD9$^{-/-}$/CD81$^{-/-}$ animals can be detected by immunohistochemistry (E. Wrobel and C. Boucheix, unpublished work).

Regulation of cell-surface expression levels

In contrast with other tetraspanins, the localization of CD63 is mostly intracellular. Found concentrated in intracellular vesicles including late endosomal compartments and lysosomes, CD63 cycles between these compartments and the plasma membrane [140]. CD63 was found to associate with the β-chain of the gastric H^+/K^+-ATPase after lysis with Triton X-100, suggesting that this pump is a CD63 partner. In unstimulated parietal cells, the H^+/K^+-ATPase resides in CD63-positive intracellular storage compartments and is translocated to the cell surface on secretagogue stimulation where it initiates acid secretion into the lumen of the stomach. The role for CD63 in regulating the subcellular localization of this pump is suggested by the fact that the HKβ subunit is present in intracellular CD63-containing vesicles when co-transfected with CD63, but localizes at the cell surface when transfected alone or co-transfected with a CD63 molecule harbouring a mutation abolishing the interaction with APs and preventing this intracellular localization [141]. An interaction of CD63 with the colonic H^+/K^+-ATPase was reported in another study in which the silencing of CD63 caused a redistribution of this pump to the plasma membrane [142]. Similarly, CD63 silencing leads to an increase in the cell-surface expression of the chemokine receptor CXCR4. Conversely, the expression of CD63, and to a greater extent the expression of a CD63 mutant molecule lacking the region upstream of the LED, led to a decrease in CXCR4 expression level at the surface [143].

Modifying the pattern of expression of tetraspanins can also have consequences on the residence time of integrins at the cell surface. Indeed, the depletion of CD151 by RNA interference leads to a lower rate of internalization of integrin $\alpha3\beta1$ [60]. In addition, mutation of the YXXΦ sorting motif in the C-terminal cytoplasmic domain of CD151 alters not only CD151 internalization and steady-state distribution, but also the rate of internalization of the fraction of integrins $\alpha3\beta1$, $\alpha6\beta1$ and $\alpha5\beta1$ linked to this tetraspanin [144]. The fact that mutation in CD151 alters the internalization of integrin $\alpha5\beta1$, which does not associate directly with CD151, suggests that the effect of this CD151 mutant on integrin internalization may be indirect. In this regard, expression of CD82, which does not interact directly with integrins, was associated with a decrease in integrin $\alpha6$ cell-surface expression and an increase in the internalization rate in prostate cancer cells [145]. It is worth noting that transfection of CD82 does not systematically decrease the surface expression level of the integrin $\alpha6\beta1$ [130].

Tetraspanins may also regulate to some extent the cell-surface expression level of membrane-anchored precursors of growth factors and cytokines. Several EGFR ligands, including HB-EGF, amphiregulin and TGFα (transforming growth factor α) have been shown to associate with CD9 which potentiates their juxtacrine activity [116,146,147]. This may be due to a change in conformation, as suggested for HB-EGF [40], or, in the case of TGFα, a stabilization of the membrane-bound growth factor precursor at the cell surface and inhibition of its cleavage [147,148]. Whether this effect is linked to the presence in the tetraspanin web of ADAM (a disintegrin and metalloproteinase) 10 [75,149], a member of the ADAM family known to cleave several EGFR ligands [150], remains to be determined.

Tetraspanins regulate proteolytic activities

MS analysis has revealed the association of tetraspanins with several membrane-bound proteases, including the serine protease matriptase/TADG-15, the ectopeptidase CD26/dipeptidyl peptidase IV and the metalloproteinase ADAM10 [47,48]. Tetraspanins may negatively regulate ADAM10 activity, since this protease is unable to cleave the membrane-anchored precursor of TNFα, while strongly associating with tetraspanins. In accordance with this, several anti-tetraspanin mAbs stimulate ADAM10-dependent cleavage of membrane-anchored precursors of TNFα and epidermal growth factor. This effect has been correlated with the ability of mAbs to induce the co-clustering of several tetraspanins and ADAM10 into membrane patches, suggesting that the mAbs modify ADAM10 activity by altering its membrane compartmentalization. ADAM10 is a partner for the tetraspanin CD53, as shown by the visualization of this complex following digitonin or Brij/EDTA lysis {a 68 kDa protein co-immunoprecipitated with CD53 [53,59] was identified as ADAM10 (F. Le Naour and E. Rubinstein, unpublished work)}.

Other studies have shown that antibodies against tetraspanins, or manipulating tetraspanin expression levels, could modify pericellular proteolytic activities. For example, CD82 expression was shown to strongly inhibit plasminogen activation as a result of decreased binding of urokinase-type plasminogen activator to its receptor [151]. This was shown to be linked to a redistribution of the receptor to focal adhesions where the receptor associated with integrin $\alpha5\beta1$, an integrin co-immunoprecipitated with tetraspanins only after lysis with the mildest detergents. Targeting tetraspanins has also been shown to modify the expression of MMPs, including MMP-2, MMP-7 and MMP-9, often at the RNA level [119,152–154]. Since integrins are known to regulate MMP production [152,155], this may reflect a modification of integrin signalling. Among the affected proteases, pro-MMP-7 may interact directly with CD151. Indeed, expression of CD151 has been shown to increase pro-MMP-7 binding and pericellular MMP-7 proteolytic activity [38]. Tetraspanins may also regulate MMP-2 activity through MT1-MMP (membrane-type matrix metalloproteinase 1), a membrane-bound metalloproteinase that associates with several tetraspanins, including CD9, CD151 and CD63 [156,157]. CD63 overexpression increases lysosomal proteolysis of MT1-MMP and consequently reduces the conversion of MMP-2 into its active form [156]. In addition, CD151 silencing increases the conversion of pro-MMP-2 and the degradation of fibrinogen, another MT1-MMP substrate, in relation to a change in MT1-MMP membrane distribution and the disruption of MT1-MMP–integrin $\alpha3\beta1$ interaction. In contrast, CD9 silencing does not affect MT1-MMP activity or membrane distribution in accordance with the tight association of MT1-MMP with CD151, but not CD9, as determined by FRET [157].

Table 2 Analysis of tetraspanin functions in whole organisms

Species	Tetraspanin	Associated phenotype	Reference
Mammals			
Human	Tspan7/TM4SF2/TALLA-1	X-linked mental retardation	[185]
	CD151	Kidney failure, pretibial bullous skin lesions, deafness, β-thalassaemia minor	[162]
	Peripherin/RDS	Retinal degeneration	Reviewed in [11]
	Rom-1	Digenic retinal degeneration	[11]
Mouse	CD9	Defects in sperm–egg fusion and paranodal junctions; increased monocyte fusion	[188,203–205,218]
	CD81	Defects of the immune response and in sperm–egg fusion; brain enlargement; increased monocyte fusion	[171,179,206,218]
	CD37	Mild alteration of the immune response	[170]
	Tspan32/Tssc6	Increased T-cell proliferation; mild platelet defect	[244,245]
	CD151	Kidney failure; altered pathological angiogenesis; delayed wound healing; abnormal platelet function; increased T-cell proliferation	[63,163,164,167,246]
	Tspan33/Penumbra	Abnormal red blood cells; anaemia	[247]
	Peripherin/RDS	Retinal degeneration	Reviewed in [11]
	Rom-1	Slow retinal degeneration	Reviewed in [11]
Amphibians			
Xenopus	Tspan-1	Abnormal gastrulation movements and neural differentiation in embryo (morpholinos)	[248]
Insects			
Drosophila melanogaster	Late bloomer	Delayed synapse formation	[176]
	Sunglasses	Light-dependent retinal degeneration	[177]
	Tsp68C	Increased expression suppresses abnormal haemocyte proliferation	[249]
Nematode worms			
Caenorhabditis elegans	TSP-15	Epidermal defects	[158]
Fungi			
Magnaporthe grisea	Pls1	Inability to penetrate into host leaves	[6]
Botrytis cinerea	Pls1	Inability to penetrate into host leaves	[7]
Podospora anserina	Pls1	Altered ascospore germination	[5]
Plants			
Arabidopsis thaliana	Ekeko/Tornado2	Altered development	[8,9]

MAJOR FUNCTIONS OF TETRASPANINS IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

Not surprisingly, considering their large distribution, tetraspanins have been implicated in quite a number of biological processes. In the following section, we review the major functions of tetraspanins at the cellular and organism levels (Table 2). Wherever possible, we indicate the relationships that could occur with known associated molecules. Having covered the unique organization of UPs in urothelial plaques, we will now see how certain tetraspanins may contribute to the generation of other discrete membrane structures that could represent highly stable and constrained modes of molecular organization.

The function of tetraspanins in epithelia

In *C. elegans*

Out of the 20 tetraspanin genes listed in wormbase, only *tsp-15* has been linked to functional defects following gene silencing. The epidermis in the nematode *C. elegans* is a classic polarized epithelium involved in the formation of the cuticle or exoskeleton of the worm. Disruption of *tsp-15* function by mutation or RNA interference results in complex defects in epidermal morphology, including a dumpy shape and blistering of the cuticle, as well as impaired barrier function. None of CD151, CD9, CD63, CD82 or Tsp74F (CG5492), a *Drosophila* tetraspanin, can rescue this *tsp-15* mutant phenotype [158]. The human tetraspanin closest to Tsp-15 is CD151 (24 % homology at the amino acid level).

In mammals

Several tetraspanins such as CD9, CD81 and CD151 are strongly expressed in various epithelia, often with a predominant localization at cell–cell contacts. In the epidermis, CD9 and CD81 are expressed in most epidermal cell layers [51,52,159], whereas CD151 expression is restricted to the basal layer, in particular at the dermal–epidermal interface, i.e. at the basal side of keratinocytes, where it most likely associates with the integrin $\alpha6\beta4$ [108,160]. Interestingly, the localization of CD151 in hemidesmosomes (stable attachments linking the basement membrane to the keratin intermediate filaments) *in vitro* is entirely dependent on the presence of this same integrin [161]. On the other hand, $CD151^{-/-}$ animals display an abnormal distribution of integrin $\alpha6\beta4$ in the skin as well as abnormal keratinocyte migration *in vitro* and delayed wound healing [139], suggesting a role for CD151 in epithelium repair.

Patients with a single nucleotide insertion in the CD151 gene leading to a translated protein lacking most of the large extracellular loop have been identified. Consistent with the presence of CD151 in hemidesmosomes, a major consequence of this mutation is restricted (pretibial) epidermolysis bullosa [162]. Mutation in human CD151 is also associated with hereditary nephritis, which can lead to end-stage renal failure. Electron microscopic examination of tissue from one patient revealed abnormalities of tubular and glomerular basement membranes [162]. Interestingly, on particular genetic backgrounds, CD151-null mice also develop renal failure with age, associated with abnormalities of tubular and glomerular basement membranes, and podocyte-specific gene deletion of the integrin $\alpha3$ subunit yields similar renal defects [163]. The authors suggested that CD151 is important for maintaining glomerular architecture upon mechanical stress, consistent with a role of CD151 in adhesion strengthening [163]. Alternatively, because thickening of the glomerular basement membrane is the first detectable morphological defect, CD151 may be involved in regulating the production, maturation or assembly of this membrane in co-operation with integrins [164].

Tetraspanin function in platelets, leucocyte extravasation and endothelial cells: a role in adhesion strengthening?

Despite the fact that CD9 is the second most abundant platelet antigen and that it associates with the fibrinogen receptor integrin αIIbβ3 [12], the function of CD9$^{-/-}$ platelets is minimally affected [165]. CD151 is expressed at a lower level and also associates with the integrin αIIbβ3 [166], an interaction that can be observed after lysis using Triton X-100 [167]. While initial platelet activation events are normal in CD151$^{-/-}$ platelets, delayed clot retraction, reduced platelet aggregation and defective spreading on fibrinogen indicate defects in post-occupancy events of integrin αIIbβ3 [167]. These results suggest that CD151 may also regulate the strengthening of integrin αIIbβ3-mediated events.

Leucocyte extravasation, a process also requiring adhesion strengthening, occurs in successive steps. Following an initial rolling phase, firm adhesion is accomplished through the interaction of integrins with vascular cell ICAM-1 and VCAM-1. Leucocyte CD81 expression confers increased resistance to detachment from VCAM-1 by incremental shear forces, strongly suggesting its enhancement of integrin $\alpha4\beta1$ adhesion strengthening. It also confers increased resistance to detachment from an integrin $\alpha5\beta1$ ligand, but not from ICAM-1, a ligand for the integrin αLβ2 [168]. Silencing of CD9 or CD151 in endothelial cells reduces the ability of lymphocytes to attach to endothelial cells, resist the detachment induced by incremental shear forces and transmigrate. These findings are consistent with the localization of CD9 and CD151 in endothelial structures containing ICAM-1 and VCAM-1 that form upon leucocyte interaction [113]. Part of this effect may be due to a decreased expression of ICAM-1 and VCAM-1. However, the partial inhibition of lymphocyte transmigration and adhesion under flow by soluble CD9 LED also suggests a direct effect of tetraspanins on the function of these molecules. Whether tetraspanins contribute directly to the formation of the docking structure remains to be determined.

None of the published tetraspanin-null mice displays vascular defects during development. However, pathological angiogenesis is impaired in CD151-null mice, and CD151-null endothelial cells show an altered behaviour in a number of tests (migration, spreading, invasion, Matrigel contraction, tube and cable formation, and spheroid sprouting) relevant to angiogenesis and in which tensional forces, and thus likely adhesion strengthening, play a critical role [63].

Tetraspanin functions in the immune system

Tetraspanins have been most extensively studied in the immune system (for reviews, see [17,112]). Deletion of tetraspanins in the mouse does not alter the distribution of lymphocyte subsets, but leads to a functional alteration of T-cells, and, in some cases, B-cells. On the other hand, several important molecules of the immune system have been shown to associate with tetraspanins including MHC class I and II antigens, CD3, CD4, CD8 and CD19 (for reviews, see [12,17,112]). With the exception of dectin-1 and CD19, whether the functional consequences of tetraspanin deletion are linked to their association with some of these molecules remains to be determined.

CD9, CD63, CD81 and CD82 have long been known to be recognized by mAbs that have a co-stimulatory activity for human and mouse T-cells. Although the simplest explanation is that tetraspanins play a positive role in T-cell activation, this hypothesis is not supported by the analysis of knockout mice. Indeed, T-cells from mice lacking CD81, CD37, CD151 or Tspan-32/TSSC6/phemx are all hyperproliferative to various *in vitro* stimulations [17,112]. Furthermore, T-cells lacking CD37 produce more IL-2 and engage faster in the first division than wild-type cells [169].

The humoral immune response of mice lacking CD9, CD151 or TSSC6 is normal [17,112]. However, CD37$^{-/-}$ mice immunized with T-dependent antigens present a diminished humoral response under conditions of suboptimal stimulation, suggesting a role of CD37 in mediating B- and T-cell interactions [170]. The role of CD81 in the humoral response is more critical, since mice lacking CD81 have a reduced Th2 cell immune response (based on the profile of cytokines released by the T-cells) following immunization with T-dependent, but not T-independent, antigens. This suggests that CD81 also plays a role in T–B-cell co-operation [171]. The presence of CD81 on both B- and T-cells is required for the normal development of a Th2 response [171,172]. CD81$^{-/-}$ B-cells show lower levels of CD19 expression [57], and CD19$^{-/-}$ mice mount a poor humoral response [136], with one report suggesting that the Th2 response may be especially affected [173]. To what extent the immune defect of CD81$^{-/-}$ mice is linked to its interaction with CD19 remains to be investigated.

Dectin-1, a major receptor of β-glucans with a prominent role in the immune response against fungi such as *Candida albicans*, interacts with the tetraspanins CD63 [174] and CD37 [175]. CD37$^{-/-}$ macrophages were shown to display a strongly increased dectin-1 ligand-induced production of IL-6, a cytokine playing an important role in host defence against fungi, despite reduced dectin-1 surface expression level and normal phagocytosis. The IL-6 production induced by LPS (lipopolysaccharide) (a Toll-like receptor 4 ligand) was unchanged [175]. How CD37 down-modulates IL-6 production induced by dectin-1 remains to be determined. It will be of special interest to study the immune response of CD37$^{-/-}$ animals to fungi.

Tetraspanins in the eye and the nervous system

In *Drosophila*

Late bloomer (lbm) is a *Drosophila* tetraspanin mainly expressed in motoneurons. The protein is present at the cell surface and is distributed along the entire length of the motor axons, including their growth cones and presynaptic terminal. In *lbm* mutant embryos, motor axons follow their appropriate pathways and normally reach their correct muscle target region by stage early 16; however, synapse formation is delayed [176]. The *lbm* gene is located on chromosome 2 within a cluster of 18 tetraspanin genes that includes two genes present on motoneurons, one of which is *sunglasses* (CG12143,Tsp42Ej). Elimination of these two genes, together with the *lbm* and six additional tetraspanin genes within the cluster, amplifies the *lbm* mutant phenotype; however, at later stages, the synapses are normal. It should be noted that removing 25 % of all fruitfly tetraspanins does not affect viability or fertility, and produces no obvious defects [3].

Sunglasses (Tsp42Ej) is a lysosomal tetraspanin predominantly expressed in the eye. The most closely related mammalian tetraspanin is CD63. Its deletion leads to fruitflies that exhibit light- and age-dependent retinal degeneration. Sunglasses associates with the G-protein-coupled light receptor rhodopsin, and regulates its trafficking to lysosomes for degradation [177,178].

Peripherin/RDS and Rom-1 play a major role in vertebrate photoreceptor architecture

The photoreceptor outer segment, specialized in the conversion of light signals into graded membrane potentials, is composed

of hundreds of stacked membranous discs that undergo daily renewal and are associated with a high density of signalling proteins [11]. The tetraspanin peripherin/RDS is proposed to play a key role in disc morphogenesis, maintenance and shedding [11]. Mutations in this protein, located at the rim of the disks, induce retinal dystrophies in human. The peripherin/RDS gene was first described in the *rds* mouse which possesses a large insertion of viral DNA resulting in a null allele. Heterozygous and homozygous animals display irregular and absent outer segments which precedes the death of photoreceptor cell bodies. Peripherin/RDS associates with a closely related tetraspanin, ROM-1. Co-inheritance of the ROM-1-null mutation has been associated with a digenic form of retinitis pigmentosa; however, monogenic mutations in this gene have no apparent pathogenic effect. Nevertheless, complete loss of this protein in knockout mice does cause outer segment shortening/disorganization and slow retinal degeneration.

CD81 regulates cell populations in the nervous system

CD81 plays a role in regulating the cellular composition of the brain. The brain of CD81$^{-/-}$ mice is larger, owing to an increase in the number of astrocytes and microglia [179]. This may be due to a loss of neuron-induced astrocyte growth arrest, as shown by *in vitro* experiments [180]. Whether CD81 acts through the binding of a ligand present on neurons, as suggested by the binding of a soluble CD81 LED molecule to neurons, remains to be determined. The role of CD81 in controlling astrocyte proliferation is supported further by experiments in which anti-CD81 mAb suppresses the mitotic activity of cultured astrocytes [181] and microglial cells [182]. Considering the secondary damage and glial scar formation in part caused by reactive glial cells during traumatic spinal cord injury, Dijkstra et al. [183] studied the effect of anti-CD81 mAb in an experimental model of this pathology and observed an enhanced functional recovery in the presence of the mAb. In addition, CD81-deficient mice have been shown to display a modified sensitivity to the neurobehavioural effects of cocaine, an effect also observed following the manipulation of CD81 expression *in vivo* using lentiviral vectors [184].

Tspan-7/TM4SF2/TALLA-1 and other tetraspanins in the nervous system

A translocation disrupting *Tspan-7/TM4SF2* identified this gene as a potential cause of mental retardation [185]. To date, the cases of X-linked mental retardation linked to this tetraspanin remain extremely rare [186]. CD9 is expressed by oligodendrocytes, and in particular on myelin, although its absence does not modify oligodendrocyte production or myelination [187]. In the peripheral nervous system, CD9 is a myelin protein important for the formation of paranodal junctions and compact myelin [188]. The function of other tetraspanins expressed in the central nervous system, including Tspan-2, Tspan-3/OAP-1, Tspan-5 and Tspan-18/neurospanin remains to be determined [12,15].

Tetraspanins in cell migration and cancer

An abundance of data based on the effect of mAbs or transfection experiments suggests a role for tetraspanins in the regulation of metastasis (for reviews, see [12,189,190]). Proposed mechanisms to explain this regulation are largely based on speculations from *in vitro* experiments using cancer cell lines. Their ability to regulate metastasis may be related to their ability to modulate migration and invasion *in vitro*, as well as to modulate growth factor signalling and integrin function, as detailed above. The ability of some to regulate proteolytic activities may also contribute towards this regulation (see above).

For the most part, tetraspanins do not appear to modulate primary tumour growth. However, the expression of Tspan-13/NET-6 in MDA-MB-231 cells was shown to inhibit the proliferation of tumour cells *in vitro* and tumour development in SCID (severe combined immunodeficient) mice [191]. Expression of CD9 in a colon cell line also led to a delay in the appearance of detectable tumour, while showing no modification of growth rate at later stages, suggesting its negative influence on initial 'grafting' [192]. Finally, silencing of CD151 in both ectopic and orthotopic xenograft models of breast cancer delays tumour formation [62].

Among the tetraspanins, CD9 and CD82 are the most extensively studied in patients. Their expression is inversely correlated with metastasis formation and bad prognosis in a large number of cancers (for reviews, see [12,189,190]). This decrease in CD82 expression may be the result of either transcriptional or post-transcriptional regulation (for a review, see [193]). Modifications of expression levels of other tetraspanins in cancer and their clinical significance have been less well characterized. A high CD151 expression level is more frequently found in high-grade tumours and correlates with poorer prognosis in colon and prostate cancer. In addition, expression levels of Co-029 are higher in poorly differentiated hepatocarcinomas as well as hepatocarcinomas with intrahepatic spreading (for reviews, see [12,189,190]).

The transfection of Co-029 in several cell types promotes metastasis, whereas that of CD9 or CD82 reduces its formation (for reviews, see [12,189,190,193]). Recent data suggest that expressing CD9 or CD82 through gene therapy may be a suitable approach to preventing the occurrence of metastasis. Indeed, the intratracheal administration of adenovirus encoding either CD9 or CD82 into lung-tumour-bearing mice reduced metastasis to the mediastinal lymph nodes [194].

Metastasis is the result of a succession of events including tissue invasion (critically modulated by adhesion molecules), entry into the circulation, arrest in distant organs and establishment of colonies in these organs [195]. The expression of tetraspanins in cancer cells was shown in several studies to reduce colony formation in the lung following intravenous injection [196,197], suggesting that tetraspanins may regulate engraftment in distant organs. In this regard, the expression of CD9 in HT-1080 cells reduced lung retention a few hours after injection. This was proposed to be due to CD9 associating with aggrus/podoplanin, a mucin-type sialoglycoprotein and ligand for the platelet CLEC-2 (C-type lectin-like receptor-2), thereby reducing the ability of the transfected cells to trigger aggrus/podoplanin-mediated platelet aggregation [198]. In another example, the expression of CD82 in BL6 melanoma cells reduced metastasis (after intravenous or subcutaneous injection) in wild-type mice, but not mice in which the *DARC* gene had been deleted [39]. This may be due to the ability of DARC, which is mainly expressed by endothelial cells, to bind to CD82, thereby reducing tumour cell growth *in vitro*, possibly as a result of increased senescence.

Investigations into the involvement of CD9 in metastasis formation began following its identification as the target of a mAb with a strong inhibitory effect on cell migration [196]. Later studies then reported similar effects on cell migration following the modification of the expression of other tetraspanins. Indeed, the transfection of CD82 and CD9 inhibited cell migration in most, if not all, reports, whereas Co-029 and CD151 transfection enhanced migration in several studies [130,131,144,154,199,200]. The positive influence of CD151 on cell migration is suggested further by the demonstration

that CD151$^{-/-}$ keratinocytes or endothelial cells, as well as CD151-silenced cells, display impaired migration on several substrates [60,62,63,201]. Importantly, modifications of CD151 expression levels affect cell migration on extracellular matrix proteins that are not ligands of the major integrins known to associate directly with CD151 ($\alpha3\beta1$, $\alpha6\beta1$, $\alpha6\beta4$) [60,144], suggesting that the mechanisms involved may not uniquely involve direct associations with these integrins. The ability of tetraspanins to modulate metastasis has always been linked to their effect on cell motility. Until recently, however, no direct evidence of a mechanism to support this had been reported. Zijlstra et al. [202] used quantitative *in vivo* assays and intravital imaging to assess the effect of an anti-metastatic anti-CD151 mAb on tumour cell migration in the chick embryo metastasis assay. In this assay, the anti-CD151 mAb had a strong inhibitory effect on metastasis, but did not inhibit tumour cell proliferation or the extravasation step. The mAb inhibited the invasion of the stroma surrounding the tumour by inhibiting detachment at the rear of the cells. The authors thus suggested that anti-cancer strategies aimed at modifying cell migration may be more efficient if the detachment step rather than the attachment step is blocked.

Tetraspanins in cell–cell fusion

The major defect observed in CD9$^{-/-}$ mice is a severe reduction in female fertility [203–205] owing to a strongly reduced ability of CD9$^{-/-}$ oocytes to fuse with sperm. Although less severe, CD81$^{-/-}$ mice also display a fertility defect, and evidence exists for CD9 and CD81 playing a complementary role in the control of sperm–egg fusion [206]. CD9 is expressed on mouse oocyte microvilli, with wild-type forms displaying a variety of shapes including long and thin, whereas CD9-null microvilli are uniformly short and thick [207]. This altered morphology may explain why CD9$^{-/-}$ oocytes are not fusion-competent. However, the fact that anti-CD9 mAbs, PSG17 and soluble CD9 and CD81 LED efficiently inhibit fertilization *in vitro* [33,110,203,208,209] suggests the contribution of other mechanisms. Interestingly, CD9 LED inhibited fusion when pre-incubated with eggs, but not with sperm, indicating that CD9 may function *in cis*, possibly by regulating the function of another oocyte surface protein [209].

CD9 associates with CD9P-1/EWI-F, EWI-2 and integrin $\alpha6\beta1$ on the egg plasma membrane [204,207]. As such, CD9 regulates the mobility of $\alpha6\beta1$ [210], an integrin once thought to play a role in sperm–egg fusion as a receptor for the sperm protein ADAM2/fertilin β [211]. Interestingly, it was demonstrated that anti-CD9 mAb inhibited the binding of eggs to beads coated with the disintegrin domain of the sperm protein ADAM2/fertilin β [208,212], but not the binding of the soluble protein [212]. This suggests that, in the presence of the anti-CD9 mAb, the fertilin β-coated beads were less able to resist to detaching shear forces during washing steps, pointing again to a role for adhesion strengthening.

Tetraspanins have been shown to modulate the differentiation of cells undergoing a fusion process. In the course of skeletal muscle differentiation, mononucleated myoblasts fuse to form multinucleated myotubes [213]. The addition of anti-CD9 or anti-CD81 antibodies delays the conversion of murine myoblastic cells into elongated myotubes, whereas ectopic expression of CD9 increases the fusion of rhabdomyosarcoma cells [214]. Macrophages can differentiate and fuse to form two types of multinucleated cell, osteoclasts and giant cells, which are important for bone resorption and the immune response respectively [215]. Silencing of Tspan-13/NET-6 increases the formation of giant multinuclear osteoclast-like cells by RANKL-treated RAW264.7 cells, whereas silencing Tspan-5 and CD9 reduces it [216,217]. CD9$^{-/-}$/CD81$^{-/-}$ mice display greater numbers of osteoclasts with reduced bone-mineral density, as well as the spontaneous appearance of multinucleated giant cells in the lung [218]. *In vitro*, the fusion of macrophages lacking these two tetraspanins is enhanced. Thus CD9 and CD81 also play a complementary role in the fusion of macrophages, and negatively regulate this process. These findings indicate a role for these tetraspanins in regulating cell fusion rather than in directly mediating membrane mixing.

Tetraspanins in infectious diseases

The role of tetraspanins in cell–cell fusion events was first made evident by the demonstration that anti-CD81 and anti-CD82 mAbs block the formation of syncitium produced by HTLV-1 (human T-lymphotropic virus 1) [219]. Similarly, anti-CD9 mAbs were subsequently demonstrated to prevent syncitium formation and/or productive infection by feline immunodeficiency virus, a cat-specific retrovirus, and canine distemper virus (for a review, see [220]). The anti-CD9 mAb blocked cell–cell fusion and virus release, without interfering with early steps including virus entry. Anti-CD9 mAb was shown recently to induce the formation of 'microvilli zippers' at cell–cell contacts from which canine distemper virus proteins were excluded [221]. Thus anti-tetraspanin mAbs may affect viral infection by modifying some properties of the plasma membrane.

HIV incorporates several tetraspanins including CD9, Tspan-14, CD81 CD82, CD53 and CD63 [222,223]. This is consistent with the fact that HIV buds in membrane domains that are enriched in several tetraspanins including CD9, CD81, CD53 and CD63 [13,224]. Anti-CD63 mAbs have been shown to strongly inhibit CCR5-mediated HIV infection of macrophages [225] and CD63 silencing to reduce HIV production [226]. However, in another study, it was reported that almost complete silencing of CD63 did not impair the formation of HIV-1 particles in human macrophages [227], suggesting a non-essential role for this tetraspanin. The enhancement of virus entry and syncytium formation by anti-CD9 and anti-CD81 mAbs provides further evidence for an indirect role of these tetraspanins in HIV infection [228].

CD81 plays a highly specific role in the entry of two major human pathogens into hepatocytes: HCV (hepatitis C virus) and the malaria parasite *Plasmodium.* In contrast, CD9 does not play a role in these two infections. Since the discovery that CD81 can bind the HCV envelope protein E2 [229], several studies have substantiated the essential role of this tetraspanin in HCV entry (for reviews, see [230,231]). The *in vitro* interaction of CD81 LED with recombinant E2 requires residues of the LED D region. Surprisingly, mutation of these residues does not abrogate the ability of CD81 molecules expressed in HepG2 cells to support HCV infection, suggesting that the interaction of CD81 with E2 may differ in a viral context from when using recombinant proteins [232]. The restricted tropism of HCV to CD81-expressing hepatocytic cells may be explained by the expression of other co-receptors, including the human scavenger receptor SR-BI and several tight junction proteins of the claudin family [230,231], or by the absence of inhibitory molecules. In this regard, the major CD81 partner EWI-2 is subjected to proteolytic cleavage in many cell types, although not hepatocytes, and transfection of this EWI-2 fragment into hepatocytic cells inhibits HCV infection to some extent [233].

Within minutes of being bitten by an infected female mosquito, the malaria parasite *Plasmodium* (at sporozoite stage) enters the liver where it invades hepatocytes and undergoes one round of multiplication. This stage is a prerequisite to the blood stages

of the life cycle which cause the malaria symptoms [234]. CD81 is the only hepatocyte surface protein known to play an essential role in infection by several *Plasmodium* species. CD81$^{-/-}$ mice are refractory to infection by *Plasmodium yoelii* sporozoites (a rodent parasite) owing to the inability of this parasite to invade CD81$^{-/-}$ hepatocytes [235]. The role of CD81 in the infection of human hepatocytes by *Plasmodium falciparum*, the deadliest human parasite, is supported by the almost complete blockade of infection *in vitro* by anti-CD81 mAbs, and reduced infection following CD81 silencing [85,235]. On the basis of the knowledge that CD9 does not support *Plasmodium* infection, the determinants of CD81 required for infection by this virus were investigated using chimaeric CD9–CD81 molecules. In this way, the role of the B helix of CD81 LED and of the residues at the junction between the A and B helices were highlighted. Surprisingly, an anti-CD81 mAb requiring the residues of this junction for optimal binding was the only anti-CD81 mAb that did not inhibit infection [236]. The simplest explanation for this paradox is that this mAb is not able to bind to the fraction of CD81 molecules competent for infection, possibly because of the association of these molecules with another surface protein concealing the mAb epitope. This has been reported for an anti-CD151 mAb not recognizing the CD151 molecules interacting with integrins [55,59].

Cholesterol depletion has been shown to reduce the infection of target cells by HIV, HCV and *Plasmodium* sporozoites [85,237–239]. Concerning HCV, this is likely to be due, at least in part, to a concomitant decrease in CD81 expression level [239]. However, the effect of cholesterol depletion on sporozoite infection was observed under conditions for which the treatment did not modify CD81 or CD9 expression levels. Whereas the treatment affects the entry of *Plasmodium* species requiring CD81 for infection, those infecting cells in a CD81-independent manner are unaffected [85]. This suggests that the effect of cholesterol depletion on *Plasmodium* infection is not the result of a general modification of properties of the plasma membrane, but is specifically linked to CD81 function. As we have seen above, this may be due to a change in the ability of CD81 to interact with other tetraspanins (including itself), possibly as a consequence of altered membrane dynamics [65,85].

CONCLUSIONS AND PERSPECTIVES

Several animal models, in mice or in invertebrates, as well as rare human diseases, have underlined the essential role played by some tetraspanins. However, gene deletions have revealed organ-restricted defects or minor phenotypic changes despite wide tissue expression. This could be due to the functional redundancy of certain tetraspanins. To investigate this further, more animal models will need to be generated and appropriate crossings made.

The field of tetraspanin research is marked by a wealth of reported molecular associations on the one hand and a poor understanding of how these interactions may support tetraspanin function on the other. Experimental data show that tetraspanins may affect trafficking, ligand binding or downstream signalling of associated molecules, but determining how they induce these changes remains a challenge. We propose that these functional effects are the consequence of a change in the ability of tetraspanins to regulate the assembly of membrane structures.

The molecular analysis of complexes containing tetraspanins has revealed preferential tetraspanin–partner interactions. However, some tetraspanins appear to have no partner, and further studies are needed to determine whether this is due to technical limits for their detection or to different functions fulfilled by these tetraspanins inside the tetraspanin web.

The recent use of advanced imaging techniques has provided the first clues to understanding the molecular dynamics within the tetraspanin web. We now know that interactions can occur both inside and outside tetraspanin-enriched structures, with rapid exchanges between the different compartments. These techniques should now lead to the further characterization of the plasticity of the tetraspanin web in both physiological and pathological conditions and help establish the relationship between molecular interactions and functional properties of tetraspanins.

ACKNOWLEDGEMENTS

Owing to the large number of published papers, we were not able to cite all individual references, and in many cases we refer the readers to reviews and references therein. We apologize to the authors whose references are not cited.

FUNDING

Our work has been supported by grants from Agence Nationale pour la Recherche, from the Groupement d'Entreprises Françaises dans la Lutte contre le Cancer Paris Ile de France, the Association pour la Recherche contre le Cancer and Nouvelles Recherches Biomédicales-Vaincre le Cancer.

REFERENCES

1 Huang, S., Yuan, S., Dong, M., Su, J., Yu, C., Shen, Y., Xie, X., Yu, Y., Yu, X., Chen, S. et al. (2005) The phylogenetic analysis of tetraspanins projects the evolution of cell–cell interactions from unicellular to multicellular organisms. Genomics **86**, 674–684
2 Todres, E., Nardi, J. B. and Robertson, H. M. (2000) The tetraspanin superfamily in insects. Insect Mol. Biol. **9**, 581–590
3 Fradkin, L. G., Kamphorst, J. T., DiAntonio, A., Goodman, C. S. and Noordermeer, J. N. (2002) Genomewide analysis of the *Drosophila* tetraspanins reveals a subset with similar function in the formation of the embryonic synapse. Proc. Natl. Acad. Sci. U.S.A. **99**, 13663–13668
4 Tran, M. H., Pearson, M. S., Bethony, J. M., Smyth, D. J., Jones, M. K., Duke, M., Don, T. A., McManus, D. P., Correa-Oliveira, R. and Loukas, A. (2006) Tetraspanins on the surface of *Schistosoma mansoni* are protective antigens against schistosomiasis. Nat. Med. **12**, 835–840
5 Lambou, K., Malagnac, F., Barbisan, C., Tharreau, D., Lebrun, M. H. and Silar, P. (2008) The crucial role during ascospore germination of the Pls1 tetraspanin in *Podospora anserina* provides an example of the convergent evolution of morphogenetic processes in fungal plant pathogens and saprobes. Eukaryotic Cell **7**, 1809–1818
6 Clergeot, P. H., Gourgues, M., Cots, J., Laurans, F., Latorse, M. P., Pepin, R., Tharreau, D., Notteghem, J. L. and Lebrun, M. H. (2001) *PLS1*, a gene encoding a tetraspanin-like protein, is required for penetration of rice leaf by the fungal pathogen *Magnaporthe grisea*. Proc. Natl. Acad. Sci. U.S.A. **98**, 6963–6968
7 Gourgues, M., Brunet-Simon, A., Lebrun, M. H. and Levis, C. (2004) The tetraspanin BcPls1 is required for appressorium-mediated penetration of *Botrytis cinerea* into host plant leaves. Mol. Microbiol. **51**, 619–629
8 Olmos, E., Reiss, B. and Dekker, K. (2003) The *ekeko* mutant demonstrates a role for tetraspanin-like protein in plant development. Biochem. Biophys. Res. Commun. **310**, 1054–1061
9 Cnops, G., Neyt, P., Raes, J., Petrarulo, M., Nelissen, H., Malenica, N., Luschnig, C., Tietz, O., Ditengou, F., Palme, K. et al. (2006) The *TORNADO1* and *TORNADO2* genes function in several patterning processes during early leaf development in *Arabidopsis thaliana*. Plant Cell **18**, 852–866
10 Sun, T. T. (2006) Altered phenotype of cultured urothelial and other stratified epithelial cells: implications for wound healing. Am. J. Physiol. Renal Physiol. **291**, F9–F21
11 Goldberg, A. F. (2006) Role of peripherin/rds in vertebrate photoreceptor architecture and inherited retinal degenerations. Int. Rev. Cytol. **253**, 131–175
12 Boucheix, C. and Rubinstein, E. (2001) Tetraspanins. Cell. Mol. Life Sci. **58**, 1189–1205
13 Deneka, M., Pelchen-Matthews, A., Byland, R., Ruiz-Mateos, E. and Marsh, M. (2007) In macrophages, HIV-1 assembles into an intracellular plasma membrane domain containing the tetraspanins CD81, CD9, and CD53. J. Cell Biol. **177**, 329–341
14 van Niel, G., Porto-Carreiro, I., Simoes, S. and Raposo, G. (2006) Exosomes: a common pathway for a specialized function. J. Biochem. **140**, 13–21
15 Hemler, M. E. (2003) Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. Annu. Rev. Cell Dev. Biol. **19**, 397–422

16 Hemler, M. E. (2005) Tetraspanin functions and associated microdomains. Nat. Rev. Mol. Cell Biol. **6**, 801–811
17 Levy, S. and Shoham, T. (2005) The tetraspanin web modulates immune-signalling complexes. Nat. Rev. Immunol. **5**, 136–148
18 Kitadokoro, K., Bordo, D., Galli, G., Petracca, R., Falugi, F., Abrignani, S., Grandi, G. and Bolognesi, M. (2001) CD81 extracellular domain 3D structure: insight into the tetraspanin superfamily structural motifs. EMBO J. **20**, 12–18
19 Min, G., Wang, H., Sun, T. T. and Kong, X. P. (2006) Structural basis for tetraspanin functions as revealed by the cryo-EM structure of uroplakin complexes at 6-Å resolution. J. Cell Biol. **173**, 975–983
20 Jones, E. Y., Davis, S. J., Williams, A. F., Harlos, K. and Stuart, D. I. (1992) Crystal structure at 2.8 Å resolution of a soluble form of the cell adhesion molecule CD2. Nature **360**, 232–239
21 Charrin, S., Manie, S., Billard, M., Ashman, L., Gerlier, D., Boucheix, C. and Rubinstein, E. (2003) Multiple levels of interactions within the tetraspanin web. Biochem. Biophys. Res. Commun. **304**, 107–112
22 Yang, X., Claas, C., Kraeft, S. K., Chen, L. B., Wang, Z., Kreidberg, J. A. and Hemler, M. E. (2002) Palmitoylation of tetraspanin proteins: modulation of CD151 lateral interactions, subcellular distribution, and integrin-dependent cell morphology. Mol. Biol. Cell **13**, 767–781
23 Zhou, B., Liu, L., Reddivari, M. and Zhang, X. A. (2004) The palmitoylation of metastasis suppressor KAI1/CD82 is important for its motility- and invasiveness-inhibitory activity. Cancer Res. **64**, 7455–7463
24 Berditchevski, F., Odintsova, E., Sawada, S. and Gilbert, E. (2002) Expression of the palmitoylation-deficient CD151 weakens the association of $\alpha3\beta1$ integrin with the tetraspanin-enriched microdomains and affects integrin-dependent signaling. J. Biol. Chem. **277**, 36991–37000
25 Levy, S., Nguyen, V. Q., Andria, M. L. and Takahashi, S. (1991) Structure and membrane topology of TAPA-1. J. Biol. Chem. **266**, 14597–14602
26 Seehafer, J. G., Tang, S. C., Slupsky, J. R. and Shaw, A. R. E. (1988) The functional glycoprotein CD9 is variably acylated: localization of the variably acylated region to a membrane-associated peptide containing the binding site for the agonistic monoclonal antibody 50H.19. Biochim. Biophys. Acta **957**, 399–410
27 Tsai, Y. C., Mendoza, A., Mariano, J. M., Zhou, M., Kostova, Z., Chen, B., Veenstra, T., Hewitt, S. M., Helman, L. J., Khanna, C. and Weissman, A. M. (2007) The ubiquitin ligase gp78 promotes sarcoma metastasis by targeting KAI1 for degradation. Nat. Med. **13**, 1504–1509
28 Lineberry, N., Su, L., Soares, L. and Fathman, C. G. (2008) The single subunit transmembrane E3 ligase gene related to anergy in lymphocytes (GRAIL) captures and then ubiquitinates transmembrane proteins across the cell membrane. J. Biol. Chem. **283**, 28497–28505
29 Seigneuret, M., Delaguillaumie, A., Lagaudriere-Gesbert, C. and Conjeaud, H. (2001) Structure of the tetraspanin main extracellular domain: a partially conserved fold with a structurally variable domain insertion. J. Biol. Chem. **276**, 40055–40064
30 Seigneuret, M. (2006) Complete predicted three-dimensional structure of the facilitator transmembrane protein and hepatitis C virus receptor CD81: conserved and variable structural domains in the tetraspanin superfamily. Biophys. J. **90**, 212–227
31 Kovalenko, O. V., Metcalf, D. G., DeGrado, W. F. and Hemler, M. E. (2005) Structural organization and interactions of transmembrane domains in tetraspanin proteins. BMC Struct. Biol. **5**, 11
32 Waterhouse, R., Ha, C. and Dveksler, G. S. (2002) Murine CD9 is the receptor for pregnancy-specific glycoprotein 17. J. Exp. Med. **195**, 277–282
33 Ellerman, D. A., Ha, C., Primakoff, P., Myles, D. G. and Dveksler, G. S. (2003) Direct binding of the ligand PSG17 to CD9 requires a CD9 site essential for sperm–egg fusion. Mol. Biol. Cell **14**, 5098–5103
34 Ha, C. T., Waterhouse, R., Wessells, J., Wu, J. A. and Dveksler, G. S. (2005) Binding of pregnancy-specific glycoprotein 17 to CD9 on macrophages induces secretion of IL-10, IL-6, PGE2, and TGF-β1. J. Leukocyte Biol. **77**, 948–957
35 Qi, J. C., Wang, J., Mandadi, S., Tanaka, K., Roufogalis, B. D., Madigan, M. C., Lai, K., Yan, F., Chong, B. H., Stevens, R. L. and Krilis, S. A. (2006) Human and mouse mast cells use the tetraspanin CD9 as an alternate interleukin-16 receptor. Blood **107**, 135–142
36 Stetler-Stevenson, W. G. (2008) Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. Sci. Signaling **1**, re6
37 Jung, K. K., Liu, X. W., Chirco, R., Fridman, R. and Kim, H. R. (2006) Identification of CD63 as a tissue inhibitor of metalloproteinase-1 interacting cell surface protein. EMBO J. **25**, 3934–3942
38 Shiomi, T., Inoki, I., Kataoka, F., Ohtsuka, T., Hashimoto, G., Nemori, R. and Okada, Y. (2005) Pericellular activation of proMMP-7 (promatrilysin-1) through interaction with CD151. Lab. Invest. **85**, 1489–1506
39 Bandyopadhyay, S., Zhan, R., Chaudhuri, A., Watabe, M., Pai, S. K., Hirota, S., Hosobe, S., Tsukada, T., Miura, K., Takano, Y. et al. (2006) Interaction of KAI1 on tumor cells with DARC on vascular endothelium leads to metastasis suppression. Nat. Med. **12**, 933–938
40 Iwamoto, R., Higashiyama, S., Mitamura, T., Taniguchi, N., Klagsbrun, M. and Mekada, E. (1994) Heparin-binding EGF-like growth factor, which acts as the diphtheria toxin receptor, forms a complex with membrane protein DRAP27/CD9, which up-regulates functional receptors and diphtheria toxin sensitivity. EMBO J. **13**, 2322–2330
41 Rubinstein, E., Le Naour, F., Lagaudrière, C., Billard, M., Conjeaud, H. and Boucheix, C. (1996) CD9, CD63, CD81 and CD82 are components of a surface tetraspan network connected to HLA-DR and VLA integrins. Eur. J. Immunol. **26**, 2657–2665
42 Berditchevski, F., Zutter, M. M. and Hemler, M. E. (1996) Characterization of novel complexes on the cell surface between integrins and proteins with 4 transmembrane domains (TM4 proteins). Mol. Biol. Cell **7**, 193–207
43 Angelisova, P., Hilgert, I. and Horejsi, V. (1994) Association of four antigens of the tetraspans family (CD37, CD53, TAPA-1, and R2/C33) with MHC class II glycoproteins. Immunogenetics **39**, 249–256
44 Little, K. D., Hemler, M. E. and Stipp, C. S. (2004) Dynamic regulation of a GPCR–tetraspanin–G protein complex on intact cells: central role of CD81 in facilitating GPR56–$G_{\alpha q/11}$ association. Mol. Biol. Cell **15**, 2375–2387
45 Stipp, C. S., Kolesnikova, T. V. and Hemler, M. E. (2001) EWI-2 is a major CD9 and CD81 partner and member of a novel Ig protein subfamily. J. Biol. Chem. **276**, 40545–40554
46 Stipp, C. S., Orlicky, D. and Hemler, M. E. (2001) FPRP, a major, highly stoichiometric, highly specific CD81- and CD9-associated protein. J. Biol. Chem. **276**, 4853–4862
47 Andre, M., Le Caer, J. P., Greco, C., Planchon, S., El Nemer, W., Boucheix, C., Rubinstein, E., Chamot-Rooke, J. and Le Naour, F. (2006) Proteomic analysis of the tetraspanin web using LC-ESI-MS/MS and MALDI-FTICR-MS. Proteomics **6**, 1437–1449
48 Le Naour, F., Andre, M., Greco, C., Billard, M., Sordat, B., Emile, J. F., Lanza, F., Boucheix, C. and Rubinstein, E. (2006) Profiling of the tetraspanin web of human colon cancer cells. Mol. Cell. Proteomics **5**, 845–857
49 Kovalenko, O. V., Yang, X. H. and Hemler, M. E. (2007) A novel cysteine cross-linking method reveals a direct association between claudin-1 and tetraspanin CD9. Mol. Cell. Proteomics **6**, 1855–1867
50 Claas, C., Stipp, C. S. and Hemler, M. E. (2001) Evaluation of prototype transmembrane 4 superfamily protein complexes and their relation to lipid rafts. J. Biol. Chem. **276**, 7974–7984
51 Yauch, R. L., Kazarov, A. R., Desai, B., Lee, R. T. and Hemler, M. E. (2000) Direct extracellular contact between integrin $\alpha3\beta1$ and TM4SF protein CD151. J. Biol. Chem. **275**, 9230–9238
52 Charrin, S., Le Naour, F., Oualid, M., Billard, M., Faure, G., Hanash, S. M., Boucheix, C. and Rubinstein, E. (2001) The major CD9 and CD81 molecular partner: identification and characterization of the complexes. J. Biol. Chem. **276**, 14329–14337
53 Charrin, S., Le Naour, F., Labas, V., Billard, M., Le Caer, J. P., Emile, J. F., Petit, M. A., Boucheix, C. and Rubinstein, E. (2003) EWI-2 is a new component of the tetraspanin web in hepatocytes and lymphoid cells. Biochem. J. **373**, 409–421
54 Lozahic, S., Christiansen, D., Manie, S., Gerlier, D., Billard, M., Boucheix, C. and Rubinstein, E. (2000) CD46 (membrane cofactor protein) associates with multiple $\beta1$ integrins and tetraspans. Eur. J. Immunol. **30**, 900–907
55 Kazarov, A. R., Yang, X., Stipp, C. S., Sehgal, B. and Hemler, M. E. (2002) An extracellular site on tetraspanin CD151 determines $\alpha3$ and $\alpha6$ integrin-dependent cellular morphology. J. Cell Biol. **158**, 1299–1309
56 Berditchevski, F., Gilbert, E., Griffiths, M. R., Fitter, S., Ashman, L. and Jenner, S. J. (2001) Analysis of the CD151–$\alpha3\beta1$ integrin and CD151–tetraspanin interactions by mutagenesis. J. Biol. Chem. **276**, 41165–41174
57 Shoham, T., Rajapaksa, R., Boucheix, C., Rubinstein, E., Poe, J. C., Tedder, T. F. and Levy, S. (2003) The tetraspanin CD81 regulates the expression of CD19 during B cell development in a postendoplasmic reticulum compartment. J. Immunol. **171**, 4062–4072
58 Tu, L., Sun, T. T. and Kreibich, G. (2002) Specific heterodimer formation is a prerequisite for uroplakins to exit from the endoplasmic reticulum. Mol. Biol. Cell **13**, 4221–4230
59 Serru, V., Le Naour, F., Billard, M., Azorsa, D. O., Lanza, F., Boucheix, C. and Rubinstein, E. (1999) Selective tetraspan/integrin complexes (CD81/$\alpha4\beta1$, CD151/$\alpha3\beta1$, CD151/$\alpha6\beta1$) under conditions disrupting tetraspan interactions. Biochem. J. **340**, 103–111
60 Winterwood, N. E., Varzavand, A., Meland, M. N., Ashman, L. K. and Stipp, C. S. (2006) A critical role for tetraspanin CD151 in $\alpha3\beta1$ and $\alpha6\beta4$ integrin-dependent tumor cell functions on laminin-5. Mol. Biol. Cell **17**, 2707–2721
61 Yamada, M., Sumida, Y., Fujibayashi, A., Fukaguchi, K., Sanzen, N., Nishiuchi, R. and Sekiguchi, K. (2008) The tetraspanin CD151 regulates cell morphology and intracellular signaling on laminin-511. FEBS J. **275**, 3335–3351

62 Yang, X. H., Richardson, A. L., Torres-Arzayus, M. I., Zhou, P., Sharma, C., Kazarov, A. R., Andzelm, M. M., Strominger, J. L., Brown, M. and Hemler, M. E. (2008) CD151 accelerates breast cancer by regulating α6 integrin function, signaling, and molecular organization. Cancer Res. **68**, 3204–3213

63 Takeda, Y., Kazarov, A. R., Butterfield, C. E., Hopkins, B. D., Benjamin, L. E., Kaipainen, A. and Hemler, M. E. (2007) Deletion of tetraspanin CD151 results in decreased pathologic angiogenesis *in vivo* and *in vitro*. Blood **109**, 1524–1532

64 Barreiro, O., Zamai, M., Yanez-Mo, M., Tejera, E., Lopez-Romero, P., Monk, P. N., Gratton, E., Caiolfa, V. R. and Sanchez-Madrid, F. (2008) Endothelial adhesion receptors are recruited to adherent leukocytes by inclusion in preformed tetraspanin nanoplatforms. J. Cell Biol. **183**, 527–542

65 Espenel, C., Margeat, E., Dosset, P., Arduise, C., Le Grimellec, C., Royer, C. A., Boucheix, C., Rubinstein, E. and Milhiet, P. E. (2008) Single-molecule analysis of CD9 dynamics and partitioning reveals multiple modes of interaction in the tetraspanin web. J. Cell Biol. **182**, 765–776

66 Kovalenko, O. V., Yang, X., Kolesnikova, T. V. and Hemler, M. E. (2004) Evidence for specific tetraspanin homodimers: inhibition of palmitoylation makes cysteine residues available for cross-linking. Biochem. J. **377**, 407–417

67 Loewen, C. J. and Molday, R. S. (2000) Disulfide-mediated oligomerization of peripherin/Rds and Rom-1 in photoreceptor disk membranes: implications for photoreceptor outer segment morphogenesis and degeneration. J. Biol. Chem. **275**, 5370–5378

68 Shoham, T., Rajapaksa, R., Kuo, C. C., Haimovich, J. and Levy, S. (2006) Building of the tetraspanin web: distinct structural domains of CD81 function in different cellular compartments. Mol. Cell. Biol. **26**, 1373–1385

69 Charrin, S., Manie, S., Thiele, C., Billard, M., Gerlier, D., Boucheix, C. and Rubinstein, E. (2003) A physical and functional link between cholesterol and tetraspanins. Eur. J. Immunol. **33**, 2479–2489

70 Charrin, S., Manie, S., Oualid, M., Billard, M., Boucheix, C. and Rubinstein, E. (2002) Differential stability of tetraspanin/tetraspanin interactions: role of palmitoylation. FEBS Lett. **516**, 139–144

71 Berquand, A., Levy, D., Gubellini, F., Le Grimellec, C. and Milhiet, P. E. (2007) Influence of calcium on direct incorporation of membrane proteins into in-plane lipid bilayer. Ultramicroscopy **107**, 928–933

72 Garidel, P. and Blume, A. (1999) Interaction of alkaline earth cations with the negatively charged phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol: a differential scanning and isothermal titration calorimetric study. Langmuir **15**, 5526–5534

73 Prachayasittikul, V., Isarankura-Na-Ayudhya, C., Tantimongcolwat, T., Nantasenamat, C. and Galla, H. J. (2007) EDTA-induced membrane fluidization and destabilization: biophysical studies on artificial lipid membranes. Acta Biochim. Biophys. Sin. (Shanghai) **39**, 901–913

74 Gambin, Y., Lopez-Esparza, R., Reffay, M., Sierecki, E., Gov, N. S., Genest, M., Hodges, R. S. and Urbach, W. (2006) Lateral mobility of proteins in liquid membranes revisited. Proc. Natl. Acad. Sci. U.S.A. **103**, 2098–2102

75 Arduise, C., Abache, T., Li, L., Billard, M., Chabanon, A., Ludwig, A., Mauduit, P., Boucheix, C., Rubinstein, E. and Le Naour, F. (2008) Tetraspanins regulate ADAM10-mediated cleavage of TNF-α and epidermal growth factor. J. Immunol. **181**, 7002–7013

76 Simons, K. and Toomre, D. (2000) Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol. **1**, 31–39

77 Munro, S. (2003) Lipid rafts: elusive or illusive? Cell **115**, 377–388

78 Delaguillaumie, A., Harriague, J., Kohanna, S., Bismuth, G., Rubinstein, E., Seigneuret, M. and Conjeaud, H. (2004) Tetraspanin CD82 controls the association of cholesterol-dependent microdomains with the actin cytoskeleton in T lymphocytes: relevance to co-stimulation. J. Cell Sci. **117**, 5269–5282

79 Claas, C., Seiter, S., Claas, A., Savelyeva, L., Schwab, M. and Zoller, M. (1998) Association between the rat homologue of CO-029, a metastasis-associated tetraspanin molecule and consumption coagulopathy. J. Cell Biol. **141**, 267–280

80 Le Naour, F., Andre, M., Boucheix, C. and Rubinstein, E. (2006) Membrane microdomains and proteomics: lessons from tetraspanin microdomains and comparison with lipid rafts. Proteomics **6**, 6447–6454

81 Ono, M., Handa, K., Sonnino, S., Withers, D. A., Nagai, H. and Hakomori, S. (2001) G_{M3} ganglioside inhibits CD9-facilitated haptotactic cell motility: coexpression of GM3 and CD9 is essential in the downregulation of tumor cell motility and malignancy. Biochemistry **40**, 6414–6421

82 Cherezov, V., Rosenbaum, D. M., Hanson, M. A., Rasmussen, S. G., Thian, F. S., Kobilka, T. S., Choi, H. J., Kuhn, P., Weis, W. I., Kobilka, B. K. and Stevens, R. C. (2007) High-resolution crystal structure of an engineered human β_2-adrenergic G protein-coupled receptor. Science **318**, 1258–1265

83 Odintsova, E., Butters, T. D., Monti, E., Sprong, H., van Meer, G. and Berditchevski, F. (2006) Gangliosides play an important role in the organization of CD82-enriched microdomains. Biochem. J. **400**, 315–325

84 Odintsova, E., Voortman, J., Gilbert, E. and Berditchevski, F. (2003) Tetraspanin CD82 regulates compartmentalisation and ligand-induced dimerization of EGFR. J. Cell Sci. **116**, 4557–4566

85 Silvie, O., Charrin, S., Billard, M., Franetich, J. F., Clark, K. L., van Gemert, G. J., Sauerwein, R. W., Dautry, F., Boucheix, C., Mazier, D. and Rubinstein, E. (2006) Cholesterol contributes to the organization of tetraspanin-enriched microdomains and to CD81-dependent infection by malaria sporozoites. J. Cell Sci. **119**, 1992–2002

86 Sharma, C., Yang, X. H. and Hemler, M. E. (2008) DHHC2 affects palmitoylation, stability, and functions of tetraspanins CD9 and CD151. Mol. Biol. Cell **19**, 3415–3425

87 Berditchevski, F. and Odintsova, E. (2007) Tetraspanins as regulators of protein trafficking. Traffic **8**, 89–96

88 Bonifacino, J. S. and Traub, L. M. (2003) Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu. Rev. Biochem. **72**, 395–447

89 Latysheva, N., Muratov, G., Rajesh, S., Padgett, M., Hotchin, N. A., Overduin, M. and Berditchevski, F. (2006) Syntenin-1 is a new component of tetraspanin-enriched microdomains: mechanisms and consequences of the interaction of syntenin-1 with CD63. Mol. Cell. Biol. **26**, 7707–7718

90 Brone, B. and Eggermont, J. (2005) PDZ proteins retain and regulate membrane transporters in polarized epithelial cell membranes. Am. J. Physiol. Cell Physiol. **288**, C20–C29

91 Pan, Y., Brown, C., Wang, X. and Geisert, E. E. (2007) The developmental regulation of CD81 in the rat retina. Mol. Vision **13**, 181–189

92 Lagaudriere-Gesbert, C., Lebel-Binay, S., Hubeau, C., Fradelizi, D. and Conjeaud, H. (1998) Signaling through the tetraspanin CD82 triggers its association with the cytoskeleton leading to sustained morphological changes and T cell activation. Eur. J. Immunol. **28**, 4332–4344

93 Delaguillaumie, A., Lagaudriere-Gesbert, C., Popoff, M. R. and Conjeaud, H. (2002) Rho GTPases link cytoskeletal rearrangements and activation processes induced via the tetraspanin CD82 in T lymphocytes. J. Cell Sci. **115**, 433–443

94 Crotta, S., Stilla, A., Wack, A., D'Andrea, A., Nuti, S., D'Oro, U., Mosca, M., Filliponi, F., Brunetto, R. M., Bonino, F. et al. (2002) Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. J. Exp. Med. **195**, 35–42

95 Crotta, S., Ronconi, V., Ulivieri, C., Baldari, C. T., Valiante, N. M., Abrignani, S. and Wack, A. (2006) Cytoskeleton rearrangement induced by tetraspanin engagement modulates the activation of T and NK cells. Eur. J. Immunol. **36**, 919–929

96 Fleming, T. J., Donnadieu, E., Song, C. H., Laethem, F. V., Galli, S. J. and Kinet, J. P. (1997) Negative regulation of FcεRI-mediated degranulation by CD81. J. Exp. Med. **186**, 1307–1314

97 Frigeri, L. and Apgar, J. R. (1999) The role of actin microfilaments in the down-regulation of the degranulation response in RBL-2H3 mast cells. J. Immunol. **162**, 2243–2250

98 Charrin, S. and Alcover, A. (2006) Role of ERM (ezrin–radixin–moesin) proteins in T lymphocyte polarization, immune synapse formation and in T cell receptor-mediated signaling. Front. Biosci. **11**, 1987–1997

99 Sala-Valdes, M., Ursa, A., Charrin, S., Rubinstein, E., Hemler, M. E., Sanchez-Madrid, F. and Yanez-Mo, M. (2006) EWI-2 and EWI-F link the tetraspanin web to the actin cytoskeleton through their direct association with ezrin–radixin–moesin proteins. J. Biol. Chem. **281**, 19665–19675

100 Force, T., Woulfe, K., Koch, W. J. and Kerkela, R. (2007) Molecular scaffolds regulate bidirectional crosstalk between Wnt and classical seven-transmembrane-domain receptor signaling pathways. Sci. STKE **2007**, e41

101 Berditchevski, F., Tolias, K. F., Wong, K., Carpenter, C. L. and Hemler, M. E. (1997) A novel link between integrins, transmembrane-4 superfamily proteins (CD63 and CD81), and phosphatidylinositol 4-kinase. J. Biol. Chem. **272**, 2595–2598

102 Zhang, X. A., Bontrager, A. L. and Hemler, M. E. (2001) Transmembrane-4 superfamily proteins associate with activated protein kinase C (PKC) and link PKC to specific β1 integrins. J. Biol. Chem. **276**, 25005–25013

103 Yauch, R. L., Berditchevski, F., Harler, M. B., Reichner, J. and Hemler, M. E. (1998) Highly stoichiometric, stable, and specific association of integrin $\alpha 3\beta 1$ with CD151 provides a major link to phosphatidylinositol 4-kinase, and may regulate cell migration. Mol. Biol. Cell **9**, 2751–2765

104 Clark, K. L., Oelke, A., Johnson, M. E., Eilert, K. D., Simpson, P. C. and Todd, S. C. (2004) CD81 associates with 14-3-3 in a redox-regulated palmitoylation-dependent manner. J. Biol. Chem. **279**, 19401–19406

105 Kolesnikova, T. V., Stipp, C. S., Rao, R. M., Lane, W. S., Luscinskas, F. W. and Hemler, M. E. (2003) EWI-2 modulates lymphocyte integrin $\alpha 4\beta 1$ functions. Blood **103**, 3013–3019

106 Unternaehrer, J. J., Chow, A., Pypaert, M., Inaba, K. and Mellman, I. (2007) The tetraspanin CD9 mediates lateral association of MHC class II molecules on the dendritic cell surface. Proc. Natl. Acad. Sci. U.S.A. **104**, 234–239

107 Zhang, X. A., Kazarov, A. R., Yang, X., Bontrager, A. L., Stipp, C. S. and Hemler, M. E. (2002) Function of the tetraspanin CD151–$\alpha 6\beta 1$ integrin complex during cellular morphogenesis. Mol. Biol. Cell **13**, 1–11

108 Chometon, G., Zhang, Z. G., Rubinstein, E., Boucheix, C., Mauch, C. and Aumailley, M. (2006) Dissociation of the complex between CD151 and laminin-binding integrins permits migration of epithelial cells. Exp. Cell Res. **312**, 983–995

109 Nishiuchi, R., Sanzen, N., Nada, S., Sumida, Y., Wada, Y., Okada, M., Takagi, J., Hasegawa, H. and Sekiguchi, K. (2005) Potentiation of the ligand-binding activity of integrin $\alpha 3\beta 1$ via association with tetraspanin CD151. Proc. Natl. Acad. Sci. U.S.A. **102**, 1939–1944

110 Higginbottom, A., Takahashi, Y., Bolling, L., Coonrod, S. A., White, J. M., Partridge, L. J. and Monk, P. N. (2003) Structural requirements for the inhibitory action of the CD9 large extracellular domain in sperm/oocyte binding and fusion. Biochem. Biophys. Res. Commun. **311**, 208–214

111 Ho, S. H., Martin, F., Higginbottom, A., Partridge, L. J., Parthasarathy, V., Moseley, G. W., Lopez, P., Cheng-Mayer, C. and Monk, P. N. (2006) Recombinant extracellular domains of tetraspanin proteins are potent inhibitors of the infection of macrophages by human immunodeficiency virus type 1. J. Virol. **80**, 6487–6496

112 Wright, M. D., Moseley, G. W. and van Spriel, A. B. (2004) Tetraspanin microdomains in immune cell signalling and malignant disease. Tissue Antigens **64**, 533–542

113 Barreiro, O., Yanez-Mo, M., Sala-Valdes, M., Gutierrez-Lopez, M. D., Ovalle, S., Higginbottom, A., Monk, P. N., Cabanas, C. and Sanchez-Madrid, F. (2005) Endothelial tetraspanin microdomains regulate leukocyte firm adhesion during extravasation. Blood **105**, 2852–2861

114 Stipp, C. S., Kolesnikova, T. V. and Hemler, M. E. (2003) EWI-2 regulates $\alpha 3\beta 1$ integrin-dependent cell functions on laminin-5. J. Cell Biol. **163**, 1167–1177

115 Yang, X. H., Kovalenko, O. V., Kolesnikova, T. V., Andzelm, M. M., Rubinstein, E., Strominger, J. L. and Hemler, M. E. (2006) Contrasting effects of EWI proteins, integrins and protein palmitoylation on cell surface CD9 organization. J. Biol. Chem. **281**, 12976–12985

116 Higashiyama, S., Iwamoto, R., Goishi, K., Raab, G., Taniguchi, N., Klagsbrun, M. and Mekada, E. (1995) The membrane protein CD9/DRAP 27 potentiates the juxtacrine growth factor activity of the membrane-anchored heparin-binding EGF-like growth factor. J. Cell Biol. **128**, 929–938

117 Hynes, R. O. (2002) Integrins: bidirectional, allosteric signaling machines. Cell **110**, 673–687

118 Lammerding, J., Kazarov, A. R., Huang, H., Lee, R. T. and Hemler, M. E. (2003) Tetraspanin CD151 regulates $\alpha 6\beta 1$ integrin adhesion strengthening. Proc. Natl. Acad. Sci. U.S.A. **100**, 7616–7621

119 Hasegawa, M., Furuya, M., Kasuya, Y., Nishiyama, M., Sugiura, T., Nikaido, T., Momota, Y., Ichinose, M. and Kimura, S. (2007) CD151 dynamics in carcinoma-stroma interaction: integrin expression, adhesion strength and proteolytic activity. Lab. Invest. **87**, 882–892

120 Murayama, Y., Miyagawa, J., Oritani, K., Yoshida, H., Yamamoto, K., Kishida, O., Miyazaki, T., Tsutsui, S., Kiyohara, T., Miyazaki, Y. et al. (2004) CD9-mediated activation of the p46 Shc isoform leads to apoptosis in cancer cells. J. Cell Sci. **117**, 3379–3388

121 Carloni, V., Mazzocca, A. and Ravichandran, K. S. (2004) Tetraspanin CD81 is linked to ERK/MAPKinase signaling by Shc in liver tumor cells. Oncogene **23**, 1566–1574

122 Horváth, G., Serru, V., Clay, D., Billard, M., Boucheix, C. and Rubinstein, E. (1998) CD19 is linked to the integrin-associated tetraspans CD9, CD81, and CD82. J. Biol. Chem. **273**, 30537–30543

123 Odintsova, E., Sugiura, T. and Berditchevski, F. (2000) Attenuation of EGF receptor signaling by a metastasis suppressor, the tetraspanin CD82/KAI-1. Curr. Biol. **10**, 1009–1012

124 Todeschini, A. R., Dos Santos, J. N., Handa, K. and Hakomori, S. I. (2007) Ganglioside G_{M2}–tetraspanin CD82 complex inhibits met and its cross-talk with integrins, providing a basis for control of cell motility through glycosynapse. J. Biol. Chem. **282**, 8123–8133

125 Wang, X. Q., Yan, Q., Sun, P., Liu, J. W., Go, L., McDaniel, S. M. and Paller, A. S. (2007) Suppression of epidermal growth factor receptor signaling by protein kinase Cα activation requires CD82, caveolin-1, and ganglioside. Cancer Res. **67**, 9986–9995

126 Takahashi, M., Sugiura, T., Abe, M., Ishii, K. and Shirasuna, K. (2007) Regulation of c-Met signaling by the tetraspanin KAI-1/CD82 affects cancer cell migration. Int. J. Cancer **121**, 1919–1929

127 Miljan, E. A. and Bremer, E. G. (2002) Regulation of growth factor receptors by gangliosides. Sci. STKE **2002**, RE15

128 Hyuga, S., Kawasaki, N., Hyuga, M., Ohta, M., Shibayama, R., Kawanishi, T., Yamagata, S., Yamagata, T. and Hayakawa, T. (2001) Ganglioside G_{D1a} inhibits HGF-induced motility and scattering of cancer cells through suppression of tyrosine phosphorylation of c-Met. Int. J. Cancer **94**, 328–334

129 Zheng, Z. Z. and Liu, Z. X. (2007) Activation of the phosphatidylinositol 3-kinase/protein kinase Akt pathway mediates CD151-induced endothelial cell proliferation and cell migration. Int. J. Biochem. Cell Biol. **39**, 340–348

130 Sridhar, S. C. and Miranti, C. K. (2006) Tetraspanin KAI1/CD82 suppresses invasion by inhibiting integrin-dependent crosstalk with c-Met receptor and Src kinases. Oncogene **25**, 2367–2378

131 Zhang, X. A., He, B., Zhou, B. and Liu, L. (2003) Requirement of the $p130^{CAS}$-Crk coupling for metastasis suppressor KAI1/CD82-mediated inhibition of cell migration. J. Biol. Chem. **278**, 27319–27328

132 Scherberich, A., Giannone, G., Perennou, E., Takeda, K., Boucheix, C., Rubinstein, E., Lanza, F. and Beretz, A. (2002) FAK-mediated inhibition of vascular smooth muscle cell migration by the tetraspanin CD9. Thromb. Haemostasis **87**, 1043–1050

133 Kotha, J., Longhurst, C., Appling, W. and Jennings, L. K. (2008) Tetraspanin CD9 regulates $\beta 1$ integrin activation and enhances cell motility to fibronectin via a PI-3 kinase-dependent pathway. Exp. Cell Res. **314**, 1811–1822

134 Saito, Y., Tachibana, I., Takeda, Y., Yamane, H., He, P., Suzuki, M., Minami, S., Kijima, T., Yoshida, M., Kumagai, T. et al. (2006) Absence of CD9 enhances adhesion-dependent morphologic differentiation, survival, and matrix metalloproteinase-2 production in small cell lung cancer cells. Cancer Res. **66**, 9557–9565

135 Kraft, S., Fleming, T., Billingsley, J. M., Lin, S. Y., Jouvin, M. H., Storz, P. and Kinet, J. P. (2005) Anti-CD63 antibodies suppress IgE-dependent allergic reactions *in vitro* and *in vivo*. J. Exp. Med. **201**, 385–396

136 Tedder, T. F., Inaoki, M. and Sato, S. (1997) The CD19-CD21 complex regulates signal transduction thresholds governing humoral immunity and autoimmunity. Immunity **6**, 107–118

137 Cherukuri, A., Cheng, P. C., Sohn, H. W. and Pierce, S. K. (2001) The CD19/CD21 complex functions to prolong B cell antigen receptor signaling from lipid rafts. Immunity **14**, 169–179

138 Cherukuri, A., Shoham, T., Sohn, H. W., Levy, S., Brooks, S., Carter, R. and Pierce, S. K. (2004) The tetraspanin CD81 is necessary for partitioning of coligated CD19/CD21-B cell antigen receptor complexes into signaling-active lipid rafts. J. Immunol. **172**, 370–380

139 Cowin, A. J., Adams, D., Geary, S. M., Wright, M. D., Jones, J. C. and Ashman, L. K. (2006) Wound healing is defective in mice lacking tetraspanin CD151. J. Invest. Dermatol. **126**, 680–689

140 Kobayashi, T., Vischer, U. M., Rosnoblet, C., Lebrand, C., Lindsay, M., Parton, R. G., Kruithof, E. K. and Gruenberg, J. (2000) The tetraspanin CD63/lamp3 cycles between endocytic and secretory compartments in human endothelial cells. Mol. Biol. Cell **11**, 1829–1843

141 Duffield, A., Kamsteeg, E. J., Brown, A. N., Pagel, P. and Caplan, M. J. (2003) The tetraspanin CD63 enhances the internalization of the H,K-ATPase β-subunit. Proc. Natl. Acad. Sci. U.S.A. **100**, 15560–15565

142 Codina, J., Li, J. and DuBose, Jr, T. D. (2005) CD63 interacts with the carboxy terminus of the colonic H^+-K^+-ATPase to decrease plasma membrane localization and $^{86}Rb^+$ uptake. Am. J. Physiol. Cell Physiol. **288**, C1279–C1286

143 Yoshida, T., Kawano, Y., Sato, K., Ando, Y., Aoki, J., Miura, Y., Komano, J., Tanaka, Y. and Koyanagi, Y. (2008) A CD63 mutant inhibits T-cell tropic human immunodeficiency virus type 1 entry by disrupting CXCR4 trafficking to the plasma membrane. Traffic **9**, 540–558

144 Liu, L., He, B., Liu, W. M., Zhou, D., Cox, J. V. and Zhang, X. A. (2007) Tetraspanin CD151 promotes cell migration by regulating integrin trafficking. J. Biol. Chem. **282**, 31631–31642

145 He, B., Liu, L., Cook, G. A., Grgurevich, S., Jennings, L. K. and Zhang, X. A. (2005) Tetraspanin CD82 attenuates cellular morphogenesis through down-regulating integrin $\alpha 6$-mediated cell adhesion. J. Biol. Chem. **280**, 3346–3354

146 Inui, S., Higashiyama, S., Hashimoto, K., Higashiyama, M., Yoshikawa, K. and Taniguchi, N. (1997) Possible role of coexpression of CD9 with membrane-anchored heparin-binding EGF-like growth factor and amphiregulin in cultured human keratinocyte growth. J. Cell. Physiol. **171**, 291–298

147 Shi, W., Fan, H., Shum, L. and Derynck, R. (2000) The tetraspanin CD9 associates with transmembrane TGF-α and regulates TGF-α-induced EGF receptor activation and cell proliferation. J. Cell Biol. **148**, 591–602

148 Imhof, I., Gasper, W. J. and Derynck, R. (2008) Association of tetraspanin CD9 with transmembrane TGFα confers alterations in cell-surface presentation of TGFα and cytoskeletal organization. J. Cell Sci. **121**, 2265–2274

149 Yan, Y., Shirakabe, K. and Werb, Z. (2002) The metalloprotease Kuzbanian (ADAM10) mediates the transactivation of EGF receptor by G protein-coupled receptors. J. Cell Biol. **158**, 221–226

150 Blobel, C. P. (2005) ADAMs: key components in EGFR signalling and development. Nat. Rev. Mol. Cell Biol. **6**, 32–43

151 Bass, R., Werner, F., Odintsova, E., Sugiura, T., Berditchevski, F. and Ellis, V. (2005) Regulation of urokinase receptor proteolytic function by the tetraspanin CD82. J. Biol. Chem. **280**, 14811–14818

152 Sugiura, T. and Berditchevski, F. (1999) Function of $\alpha 3\beta 1$–tetraspanin protein complexes in tumor cell invasion: evidence for the role of the complexes in production of matrix metalloproteinase 2 (MMP-2). J. Cell Biol. **146**, 1375–1389

153 Hong, I. K., Jin, Y. J., Byun, H. J., Jeoung, D. I., Kim, Y. M. and Lee, H. (2006) Homophilic interactions of tetraspanin CD151 up-regulate motility and matrix metalloproteinase-9 expression of human melanoma cells through adhesion-dependent c-Jun activation signaling pathways. J. Biol. Chem. **281**, 24279–24292
154 Zhou, Z., Ran, Y. L., Hu, H., Pan, J., Li, Z. F., Chen, L. Z., Sun, L. C., Peng, L., Zhao, X. L., Yu, L. et al. (2008) TM4SF3 promotes esophageal carcinoma metastasis via upregulating ADAM12m expression. Clin. Exp. Metastasis **25**, 537–548
155 Munshi, H. G. and Stack, M. S. (2006) Reciprocal interactions between adhesion receptor signaling and MMP regulation. Cancer Metastasis Rev. **25**, 45–56
156 Takino, T., Miyamori, H., Kawaguchi, N., Uekita, T., Seiki, M. and Sato, H. (2003) Tetraspanin CD63 promotes targeting and lysosomal proteolysis of membrane-type 1 matrix metalloproteinase. Biochem. Biophys. Res. Commun. **304**, 160–166
157 Yanez-Mo, M., Barreiro, O., Gonzalo, P., Batista, A., Megias, D., Genis, L., Sachs, N., Sala-Valdes, M., Alonso, M. A., Montoya, M. C. et al. (2008) MT1-MMP collagenolytic activity is regulated through association with tetraspanin CD151 in primary endothelial cells. Blood **112**, 3217–3226
158 Moribe, H., Yochem, J., Yamada, H., Tabuse, Y., Fujimoto, T. and Mekada, E. (2004) Tetraspanin protein (TSP-15) is required for epidermal integrity in *Caenorhabditis elegans*. J. Cell Sci. **117**, 5209–5220
159 Okochi, H., Kato, M., Nashiro, K., Yoshie, O., Miyazono, K. and Furue, M. (1997) Expression of tetra-spans transmembrane family (CD9, CD37, CD53, CD63, CD81 and CD82) in normal and neoplastic human keratinocytes: an association of CD9 with $\alpha 3\beta 1$ integrin. Br. J. Dermatol. **137**, 856–863
160 Sterk, L. M., Geuijen, C. A., van den Berg, J. G., Claessen, N., Weening, J. J. and Sonnenberg, A. (2002) Association of the tetraspanin CD151 with the laminin-binding integrins $\alpha 3\beta 1$, $\alpha 6\beta 1$, $\alpha 6\beta 4$ and $\alpha 7\beta 1$ in cells in culture and *in vivo*. J. Cell Sci. **115**, 1161–1173
161 Sterk, L. M., Geuijen, C. A., Oomen, L. C., Calafat, J., Janssen, H. and Sonnenberg, A. (2000) The tetraspan molecule CD151, a novel constituent of hemidesmosomes, associates with the integrin $\alpha 6\beta 4$ and may regulate the spatial organization of hemidesmosomes. J. Cell Biol. **149**, 969–982
162 Karamatic Crew, V., Burton, N., Kagan, A., Green, C. A., Levene, C., Flinter, F., Brady, R. L., Daniels, G. and Anstee, D. J. (2004) CD151, the first member of the tetraspanin (TM4) superfamily detected on erythrocytes, is essential for the correct assembly of human basement membranes in kidney and skin. Blood **104**, 2217–2223
163 Sachs, N., Kreft, M., van den Bergh Weerman, MA, Beynon, A. J., Peters, T. A., Weening, J. J. and Sonnenberg, A. (2006) Kidney failure in mice lacking the tetraspanin CD151. J. Cell Biol. **175**, 33–39
164 Baleato, R. M., Guthrie, P. L., Gubler, M. C., Ashman, L. K. and Roselli, S. (2008) Deletion of CD151 results in a strain-dependent glomerular disease due to severe alterations of the glomerular basement membrane. Am. J. Pathol. **173**, 927–937
165 Mangin, P. H., Kleitz, L., Boucheix, C., Gachet, C. and Lanza, F. (2009) CD9 negatively regulates integrin αIIb$\beta 3$ activation and could thus prevent excessive platelet recruitment at sites of vascular injury, J. Thromb. Haemostasis, doi:10.1111/j.1538-7836.2009.03322.x
166 Fitter, S., Sincock, P. M., Jolliffe, C. N. and Ashman, L. K. (1999) Transmembrane 4 superfamily protein CD151 (PETA-3) associates with $\beta 1$ and αIIb$\beta 3$ integrins in haemopoietic cell lines and modulates cell–cell adhesion. Biochem. J. **338**, 61–70
167 Lau, L. M., Wee, J. L., Wright, M. D., Moseley, G. W., Hogarth, P. M., Ashman, L. K. and Jackson, D. E. (2004) The tetraspanin superfamily member CD151 regulates outside-in integrin αIIb$\beta 3$ signaling and platelet function. Blood **104**, 2368–2375
168 Feigelson, S. W., Grabovsky, V., Shamri, R., Levy, S. and Alon, R. (2003) The CD81 tetraspanin facilitates instantaneous leukocyte VLA-4 adhesion strengthening to vascular cell adhesion molecule 1 (VCAM-1) under shear flow. J. Biol. Chem. **278**, 51203–51212
169 van Spriel, A. B., Puls, K. L., Sofi, M., Pouniotis, D., Hochrein, H., Orinska, Z., Knobeloch, K. P., Plebanski, M. and Wright, M. D. (2004) A regulatory role for CD37 in T cell proliferation. J. Immunol. **172**, 2953–2961
170 Knobeloch, K. P., Wright, M. D., Ochsenbein, A. F., Liesenfeld, O., Lohler, J., Zinkernagel, R. M., Horak, I. and Orinska, Z. (2000) Targeted inactivation of the tetraspanin CD37 impairs T-cell-dependent B-cell response under suboptimal costimulatory conditions. Mol. Cell Biol. **20**, 5363–5369
171 Maecker, H. T., Do, M. S. and Levy, S. (1998) CD81 on B cells promotes interleukin 4 secretion and antibody production during T helper type 2 immune responses. Proc. Natl. Acad. Sci. U.S.A. **95**, 2458–2462
172 Deng, J., DeKruyff, R. H., Freeman, G. J., Umetsu, D. T. and Levy, S. (2002) Critical role of CD81 in cognate T–B cell interactions leading to Th2 responses. Int. Immunol. **14**, 513–523
173 Gardby, E., Chen, X. J. and Lycke, N. Y. (2001) Impaired CD40-signalling in CD19-deficient mice selectively affects Th2-dependent isotype switching. Scand. J. Immunol. **53**, 13–23
174 Mantegazza, A. R., Barrio, M. M., Moutel, S., Bover, L., Weck, M., Brossart, P., Teillaud, J. L. and Mordoh, J. (2004) CD63 tetraspanin slows down cell migration and translocates to the endosomal-lysosomal-MIICs route after extracellular stimuli in human immature dendritic cells. Blood **104**, 1183–1190
175 Meyer-Wentrup, F., Figdor, C. G., Ansems, M., Brossart, P., Wright, M. D., Adema, G. J. and van Spriel, A. B. (2007) Dectin-1 interaction with tetraspanin CD37 inhibits IL-6 production. J. Immunol. **178**, 154–162
176 Kopczynski, C. C., Davis, G. W. and Goodman, C. S. (1996) A neural tetraspanin, encoded by late bloomer, that facilitates synapse formation. Science **271**, 1867–1870
177 Xu, H., Lee, S. J., Suzuki, E., Dugan, K. D., Stoddard, A., Li, H. S., Chodosh, L. A. and Montell, C. (2004) A lysosomal tetraspanin associated with retinal degeneration identified via a genome-wide screen. EMBO J. **23**, 811–822
178 Han, J., Reddig, K. and Li, H. S. (2007) Prolonged G_q activity triggers fly rhodopsin endocytosis and degradation, and reduces photoreceptor sensitivity. EMBO J. **26**, 4966–4973
179 Geisert, Jr, E. E., Williams, R. W., Geisert, G. R., Fan, L., Asbury, A. M., Maecker, H. T., Deng, J. and Levy, S. (2002) Increased brain size and glial cell number in CD81-null mice. J. Comp. Neurol. **453**, 22–32
180 Kelic, S., Levy, S., Suarez, C. and Weinstein, D. E. (2001) CD81 regulates neuron-induced astrocyte cell-cycle exit. Mol. Cell. Neurosci. **17**, 551–560
181 Geisert, E. E. j., Yang, L. and Irwin, M. H. (1996) Astrocyte growth, reactivity, and the target of the antiproliferative antibody, TAPA. J. Neurosci. **16**, 5478–5487
182 Dijkstra, S., Geisert, Jr, E. E., Dijkstra, C. D., Bar, P. R. and Joosten, E. A. (2001) CD81 and microglial activation *in vitro*: proliferation, phagocytosis and nitric oxide production. J. Neuroimmunol. **114**, 151–159
183 Dijkstra, S., Duis, S., Pans, I. M., Lankhorst, A. J., Hamers, F. P., Veldman, H., Bar, P. R., Gispen, W. H., Joosten, E. A. and Geisert, Jr, E. E. (2006) Intraspinal administration of an antibody against CD81 enhances functional recovery and tissue sparing after experimental spinal cord injury. Exp. Neurol. **202**, 57–66
184 Bahi, A., Boyer, F., Kolira, M. and Dreyer, J. L. (2005) *In vivo* gene silencing of CD81 by lentiviral expression of small interference RNAs suppresses cocaine-induced behaviour. J. Neurochem. **92**, 1243–1255
185 Zemni, R., Bienvenu, T., Vinet, M. C., Sefiani, A., Carrie, A., Billuart, P., McDonell, N., Couvert, P., Francis, F., Chafey, P. et al. (2000) A new gene involved in X-linked mental retardation identified by analysis of an X;2 balanced translocation. Nat. Genet. **24**, 167–170
186 Raymond, F. L. (2006) X linked mental retardation: a clinical guide. J. Med. Genet. **43**, 193–200
187 Terada, N., Baracskay, K., Kinter, M., Melrose, S., Brophy, P. J., Boucheix, C., Bjartmar, C., Kidd, G. and Trapp, B. D. (2002) The tetraspanin protein, CD9, is expressed by progenitor cells committed to oligodendrogenesis and is linked to $\beta 1$ integrin, CD81, and Tspan-2. Glia **40**, 350–359
188 Ishibashi, T., Ding, L., Ikenaka, K., Inoue, Y., Miyado, K., Mekada, E. and Baba, H. (2004) Tetraspanin protein CD9 is a novel paranodal component regulating paranodal junctional formation. J. Neurosci. **24**, 96–102
189 Lazo, P. A. (2007) Functional implications of tetraspanin proteins in cancer biology. Cancer Sci. **98**, 1666–1677
190 Zoller, M. (2009) Tetraspanins: push and pull in suppressing and promoting metastasis. Nat. Rev. Cancer **9**, 40–55
191 Huang, H., Sossey-Alaoui, K., Beachy, S. H. and Geradts, J. (2007) The tetraspanin superfamily member NET-6 is a new tumor suppressor gene. J. Cancer Res. Clin. Oncol. **133**, 761–769
192 Ovalle, S., Gutierrez-Lopez, M. D., Olmo, N., Turnay, J., Lizarbe, M. A., Majano, P., Molina-Jimenez, F., Lopez-Cabrera, M., Yanez-Mo, M., Sanchez-Madrid, F. and Cabanas, C. (2007) The tetraspanin CD9 inhibits the proliferation and tumorigenicity of human colon carcinoma cells. Int. J. Cancer **121**, 2140–2152
193 Liu, W. M. and Zhang, X. A. (2006) KAI1/CD82, a tumor metastasis suppressor. Cancer Lett. **240**, 183–194
194 Takeda, T., Hattori, N., Tokuhara, T., Nishimura, Y., Yokoyama, M. and Miyake, M. (2007) Adenoviral transduction of MRP-1/CD9 and KAI1/CD82 inhibits lymph node metastasis in orthotopic lung cancer model. Cancer Res. **67**, 1744–1749
195 Fidler, I. J. (2003) The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat. Rev. Cancer **3**, 453–458
196 Ikeyama, S., Koyama, M., Yamaoko, M., Sasada, R. and Miyake, M. (1993) Suppression of cell motility and metastasis by transfection with human motility-related protein (MRP-1/CD9) DNA. J. Exp. Med. **177**, 1231–1237
197 Yang, X., Wei, L. L., Tang, C., Slack, R., Mueller, S. and Lippman, M. E. (2001) Overexpression of KAI1 suppresses *in vitro* invasiveness and *in vivo* metastasis in breast cancer cells. Cancer Res. **61**, 5284–5288
198 Nakazawa, Y., Sato, S., Naito, M., Kato, Y., Mishima, K., Arai, H., Tsuruo, T. and Fujita, N. (2008) Tetraspanin family member CD9 inhibits aggrus/podoplanin-induced platelet aggregation and suppresses pulmonary metastasis. Blood **112**, 1730–1739

199 Testa, J. E., Brooks, P. C., Lin, J. M. and Quigley, J. P. (1999) Eukaryotic expression cloning with an antimetastatic monoclonal antibody identifies a tetraspanin (PETA-3/CD151) as an effector of human tumor cell migration and metastasis. Cancer Res. **59**, 3812–3820

200 Shigeta, M., Sanzen, N., Ozawa, M., Gu, J., Hasegawa, H. and Sekiguchi, K. (2003) CD151 regulates epithelial cell–cell adhesion through PKC- and Cdc42-dependent actin cytoskeletal reorganization. J. Cell Biol. **163**, 165–176

201 Geary, S. M., Cowin, A. J., Copeland, B., Baleato, R. M., Miyazaki, K. and Ashman, L. K. (2008) The role of the tetraspanin CD151 in primary keratinocyte and fibroblast functions: implications for wound healing. Exp. Cell Res. **314**, 2165–2175

202 Zijlstra, A., Lewis, J., Degryse, B., Stuhlmann, H. and Quigley, J. P. (2008) The inhibition of tumor cell intravasation and subsequent metastasis via regulation of *in vivo* tumor cell motility by the tetraspanin CD151. Cancer Cell **13**, 221–234

203 Le Naour, F., Rubinstein, E., Jasmin, C., Prenant, M. and Boucheix, C. (2000) Severely reduced female fertility in CD9-deficient mice. Science **287**, 319–321

204 Miyado, K., Yamada, G., Yamada, S., Hasuwa, H., Nakamura, Y., Ryu, F., Suzuki, K., Kosai, K., Inoue, K., Ogura, A. et al. (2000) Requirement of CD9 on the egg plasma membrane for fertilization. Science **287**, 321–324

205 Kaji, K., Oda, S., Shikano, T., Ohnuki, T., Uematsu, Y., Sakagami, J., Tada, N., Miyazaki, S. and Kudo, A. (2000) The gamete fusion process is defective in eggs of CD9-deficient mice. Nat. Genet. **24**, 279–282

206 Rubinstein, E., Ziyyat, A., Prenant, M., Wrobel, E., Wolf, J. P., Levy, S., Le Naour, F. and Boucheix, C. (2006) Reduced fertility of female mice lacking CD81. Dev. Biol. **290**, 351–358

207 Runge, K. E., Evans, J. E., He, Z. Y., Gupta, S., McDonald, K. L., Stahlberg, H., Primakoff, P. and Myles, D. G. (2007) Oocyte CD9 is enriched on the microvillar membrane and required for normal microvillar shape and distribution. Dev. Biol. **304**, 317–325

208 Chen, M. S., Tung, K. S., Coonrod, S. A., Takahashi, Y., Bigler, D., Chang, A., Yamashita, Y., Kincade, P. W., Herr, J. C. and White, J. M. (1999) Role of the integrin-associated protein CD9 in binding between sperm ADAM 2 and the egg integrin $\alpha 6\beta 1$: implications for murine fertilization. Proc. Natl. Acad. Sci. U.S.A. **96**, 11830–11835

209 Zhu, G. Z., Miller, B. J., Boucheix, C., Rubinstein, E., Liu, C. C., Hynes, R. O., Myles, D. G. and Primakoff, P. (2002) Residues SFQ (173–175) in the large extracellular loop of CD9 are required for gamete fusion. Development **129**, 1995–2002

210 Ziyyat, A., Rubinstein, E., Monier-Gavelle, F., Barraud, V., Kulski, O., Prenant, M., Boucheix, C., Bomsel, M. and Wolf, J. P. (2006) CD9 controls the formation of clusters that contain tetraspanins and the integrin $\alpha 6\beta 1$, which are involved in human and mouse gamete fusion. J. Cell Sci. **119**, 416–424

211 Rubinstein, E., Ziyyat, A., Wolf, J. P., Le Naour, F. and Boucheix, C. (2006) The molecular players of sperm–egg fusion in mammals. Semin. Cell Dev. Biol. **17**, 254–263

212 Zhu, X. and Evans, J. P. (2002) Analysis of the roles of RGD-binding integrins, $\alpha 4/\alpha 9$ integrins, $\alpha 6$ integrins, and CD9 in the interaction of the fertilin β (ADAM2) disintegrin domain with the mouse egg membrane. Biol. Reprod. **66**, 1193–1202

213 Chen, E. H., Grote, E., Mohler, W. and Vignery, A. (2007) Cell–cell fusion. FEBS Lett. **581**, 2181–2193

214 Tachibana, I. and Hemler, M. E. (1999) Role of transmembrane 4 superfamily (TM4SF) proteins CD9 and CD81 in muscle cell fusion and myotube maintenance. J. Cell Biol. **146**, 893–904

215 Vignery, A. (2005) Macrophage fusion: the making of osteoclasts and giant cells. J. Exp. Med. **202**, 337–340

216 Ishii, M., Iwai, K., Koike, M., Ohshima, S., Kudo-Tanaka, E., Ishii, T., Mima, T., Katada, Y., Miyatake, K., Uchiyama, Y. and Saeki, Y. (2006) RANKL-induced expression of tetraspanin CD9 in lipid raft membrane microdomain is essential for cell fusion during osteoclastogenesis. J. Bone Miner. Res. **21**, 965–976

217 Iwai, K., Ishii, M., Ohshima, S., Miyatake, K. and Saeki, Y. (2007) Expression and function of transmembrane-4 superfamily (tetraspanin) proteins in osteoclasts: reciprocal roles of Tspan-5 and NET-6 during osteoclastogenesis. Allergol. Int. **56**, 457–463

218 Takeda, Y., Tachibana, I., Miyado, K., Kobayashi, M., Miyazaki, T., Funakoshi, T., Kimura, H., Yamane, H., Saito, Y., Goto, H. et al. (2003) Tetraspanins CD9 and CD81 function to prevent the fusion of mononuclear phagocytes. J. Cell Biol. **161**, 945–956

219 Imai, T. and Yoshie, O. (1993) C33 antigen and M38 antigen recognized by monoclonal antibodies inhibitory to syncytium formation by human T cell leukemia virus type 1 are both members of the transmembrane 4 superfamily and associate with each other and with CD4 or CD8 in T cells. J. Immunol. **151**, 6470–6481

220 Martin, F., Roth, D. M., Jans, D. A., Pouton, C. W., Partridge, L. J., Monk, P. N. and Moseley, G. W. (2005) Tetraspanins in viral infections: a fundamental role in viral biology? J. Virol. **79**, 10839–10851

221 Singethan, K., Muller, N., Schubert, S., Luttge, D., Krementsov, D. N., Khurana, S. R., Krohne, G., Schneider-Schaulies, S., Thali, M. and Schneider-Schaulies, J. (2008) CD9 clustering and formation of microvilli zippers between contacting cells regulates virus-induced cell fusion. Traffic **9**, 924–935

222 Pelchen-Matthews, A., Kramer, B. and Marsh, M. (2003) Infectious HIV-1 assembles in late endosomes in primary macrophages. J. Cell Biol. **162**, 443–455

223 Chertova, E., Chertov, O., Coren, L. V., Roser, J. D., Trubey, C. M., Bess, Jr, J. W., Sowder, R. C., Barsov, E., Hood, B. L., Fisher, R. J. et al. (2006) Proteomic and biochemical analysis of purified human immunodeficiency virus type 1 produced from infected monocyte-derived macrophages. J. Virol. **80**, 9039–9052

224 Nydegger, S., Khurana, S., Krementsov, D. N., Foti, M. and Thali, M. (2006) Mapping of tetraspanin-enriched microdomains that can function as gateways for HIV-1. J. Cell Biol. **173**, 795–807

225 von Lindern, J. J., Rojo, D., Grovit-Ferbas, K., Yeramian, C., Deng, C., Herbein, G., Ferguson, M. R., Pappas, T. C., Decker, J. M., Singh, A. et al. (2003) Potential role for CD63 in CCR5-mediated human immunodeficiency virus type 1 infection of macrophages. J. Virol. **77**, 3624–3633

226 Chen, H., Dziuba, N., Friedrich, B., von Lindern, J., Murray, J. L., Rojo, D. R., Hodge, T. W., O'Brien, W. A. and Ferguson, M. R. (2008) A critical role for CD63 in HIV replication and infection of macrophages and cell lines. Virology **379**, 191–196

227 Ruiz-Mateos, E., Pelchen-Matthews, A., Deneka, M. and Marsh, M. (2008) CD63 is not required for production of infectious human immunodeficiency virus type 1 in human macrophages. J. Virol. **82**, 4751–4761

228 Gordon-Alonso, M., Yanez-Mo, M., Barreiro, O., Alvarez, S., Munoz-Fernandez, M. A., Valenzuela-Fernandez, A. and Sanchez-Madrid, F. (2006) Tetraspanins CD9 and CD81 modulate HIV-1-induced membrane fusion. J. Immunol. **177**, 5129–5137

229 Pileri, P., Uematsu, Y., Campagnoli, S., Galli, G., Falugi, F., Petracca, R., Weiner, A. J., Houghton, M., Rosa, D., Grandi, G. and Abrignani, S. (1998) Binding of hepatitis C virus to CD81. Science **282**, 938–941

230 Stamataki, Z., Grove, J., Balfe, P. and McKeating, J. A. (2008) Hepatitis C virus entry and neutralization. Clin. Liver Dis. **12**, 693–712

231 Cocquerel, L., Voisset, C. and Dubuisson, J. (2006) Hepatitis C virus entry: potential receptors and their biological functions. J. Gen. Virol. **87**, 1075–1084

232 Zhang, J., Randall, G., Higginbottom, A., Monk, P., Rice, C. M. and McKeating, J. A. (2004) CD81 is required for hepatitis C virus glycoprotein-mediated viral infection. J. Virol. **78**, 1448–1455

233 Rocha-Perugini, V., Montpellier, C., Delgrange, D., Wychowski, C., Helle, F., Pillez, A., Drobecq, H., Le Naour, F., Charrin, S., Levy, S. et al. (2008) The CD81 partner EWI-2^{wint} inhibits hepatitis C virus entry. PLoS ONE **3**, e1866

234 Silvie, O., Mota, M. M., Matuschewski, K. and Prudencio, M. (2008) Interactions of the malaria parasite and its mammalian host. Curr. Opin. Microbiol. **11**, 352–359

235 Silvie, O., Rubinstein, E., Franetich, J. F., Prenant, M., Belnoue, E., Renia, L., Hannoun, L., Eling, W., Levy, S., Boucheix, C. and Mazier, D. (2003) Hepatocyte CD81 is required for *Plasmodium falciparum* and *Plasmodium yoelii* sporozoite infectivity. Nat. Med. **9**, 93–96

236 Yalaoui, S., Zougbede, S., Charrin, S., Silvie, O., Arduise, C., Farhati, K., Boucheix, C., Mazier, D., Rubinstein, E. and Froissard, P. (2008) Hepatocyte permissiveness to *Plasmodium* infection is conveyed by a short and structurally conserved region of the CD81 large extracellular domain. PLoS Pathog. **4**, e1000010

237 Liao, Z., Cimakasky, L. M., Hampton, R., Nguyen, D. H. and Hildreth, J. E. (2001) Lipid rafts and HIV pathogenesis: host membrane cholesterol is required for infection by HIV type 1. AIDS Res. Hum. Retroviruses **17**, 1009–1019

238 Popik, W., Alce, T. M. and Au, W. C. (2002) Human immunodeficiency virus type 1 uses lipid raft-colocalized CD4 and chemokine receptors for productive entry into CD4$^+$ T cells. J. Virol. **76**, 4709–4722

239 Kapadia, S. B., Barth, H., Baumert, T., McKeating, J. A. and Chisari, F. V. (2007) Initiation of hepatitis C virus infection is dependent on cholesterol and cooperativity between CD81 and scavenger receptor B type I. J. Virol. **81**, 374–383

240 Bradbury, L. E., Kansas, G. S., Levy, S., Evans, R. L. and Tedder, T. F. (1992) The CD19/CD21 signal transducing complex of human B lymphocytes includes the target of antiproliferative antibody-1 and Leu-13 molecules. J. Immunol. **149**, 2841–2850

241 Matsumoto, A. K., Martin, D. R., Carter, R. H., Klickstein, L. B., Ahearn, J. M. and Fearon, D. T. (1993) Functional dissection of the CD21/CD19/TAPA-1/leu-13 complex of B lymphocytes. J. Exp. Med. **178**, 1407–1417

242 Clark, K. L., Zeng, Z., Langford, A. L., Bowen, S. M. and Todd, S. C. (2001) Pgrl is a major CD81-associated protein on lymphocytes and distinguishes a new family of cell surface proteins. J. Immunol. **167**, 5115–5121

243 Harris, H. J., Farquhar, M. J., Mee, C. J., Davis, C., Reynolds, G. M., Jennings, A., Hu, K., Yuan, F., Deng, H., Hubscher, S. G. et al. (2008) CD81 and claudin 1 coreceptor association: role in hepatitis C virus entry. J. Virol. **82**, 5007–5020

244 Tarrant, J. M., Groom, J., Metcalf, D., Li, R., Borobokas, B., Wright, M. D., Tarlinton, D. and Robb, L. (2002) The absence of Tssc6, a member of the tetraspanin superfamily, does not affect lymphoid development but enhances *in vitro* T-cell proliferative responses. Mol. Cell. Biol. **22**, 5006–5018

245 Goschnick, M. W., Lau, L. M., Wee, J. L., Liu, Y. S., Hogarth, P. M., Robb, L. M., Hickey, M. J., Wright, M. D. and Jackson, D. E. (2006) Impaired "outside-in" integrin αIIbβ3 signaling and thrombus stability in TSSC6-deficient mice. Blood **108**, 1911–1918

246 Wright, M. D., Geary, S. M., Fitter, S., Moseley, G. W., Lau, L. M., Sheng, K. C., Apostolopoulos, V., Stanley, E. G., Jackson, D. E. and Ashman, L. K. (2004) Characterization of mice lacking the tetraspanin superfamily member CD151. Mol. Cell. Biol. **24**, 5978–5988

247 Heikens, M. J., Cao, T. M., Morita, C., Dehart, S. L. and Tsai, S. (2007) Penumbra encodes a novel tetraspanin that is highly expressed in erythroid progenitors and promotes effective erythropoiesis. Blood **109**, 3244–3252

248 Yamamoto, Y., Grubisic, K. and Oelgeschlager, M. (2007) *Xenopus* tetraspanin-1 regulates gastrulation movements and neural differentiation in the early *Xenopus* embryo. Differentiation **75**, 235–245

249 Sinenko, S. A. and Mathey-Prevot, B. (2004) Increased expression of *Drosophila* tetraspanin, Tsp68C, suppresses the abnormal proliferation of ytr-deficient and Ras/Raf-activated hemocytes. Oncogene **23**, 9120–9128

Received 19 December 2008/17 March 2009; accepted 19 March 2009
Published on the Internet 13 May 2009, doi:10.1042/BJ20082422