Complexes of tetraspanins with integrins: more than meets the eye

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Summary

The transmembrane proteins of the tetraspanin superfamily are implicated in a diverse range of biological phenomena, including cell motility, metastasis, cell proliferation and differentiation. The tetraspanins are associated with adhesion receptors of the integrin family and regulate integrin-dependent cell migration. In cells attached to the extracellular matrix, the integrin-tetraspanin adhesion complexes are clustered into a distinct type of adhesion structure at the cell periphery. Various tetraspanins are associated with phosphatidylinositol 4-kinase and protein kinase C isoforms, and they may facilitate assembly of signalling complexes by tethering these enzymes to integrin heterodimers. At the plasma membrane, integrin-tetraspanin signalling complexes are partitioned into specific microdomains proximal to cholesterol-rich lipid rafts. A substantial fraction of tetraspanins colocalise with integrins in various intracellular vesicular compartments. It is proposed that tetraspanins can influence cell migration by one of the following mechanisms: (1) modulation of integrin signalling; (2) compartmentalisation of integrins on the cell surface; or (3) direction of intracellular trafficking and recycling of integrins.

Key words: Tetraspanin, Integrin, Migration, Signalling

Introduction

Tetraspanins (also referred to as tetraspans or TM4SF proteins) are a family of widely expressed four-transmembrane-domain proteins. Tetraspanins first appeared in multicellular organisms (the sponge genome contains at least two tetraspanin genes), and the family contains more >26 proteins* in higher eukaryotic organisms. Tetraspanins are postulated to have a topology in which there are two extracellular loops (a small loop between the first and second transmembrane (TM) domains, and a large loop between the third and forth TM domains), an interconnecting intracellular loop (between the second and third TM domains), and cytoplasmic N- and C-termini (Fig. 1). Although the external location of the extracellular loops has been confirmed experimentally (Jennings et al., 1994; Levy et al., 1991), the cytoplasmic location of the four consecutive hydrophilic residues of the interconnecting loop remains a point of speculation.

A hallmark of the tetraspanin superfamily is the presence of a Cys-Cys-Gly sequence (the CCG motif) within the large extracellular loop (LECL) of the protein. In addition, the LECLs of most tetraspanins contain two or four additional cysteine residues, one of which is placed 11 residues from the predicted start of the fourth TM domain†. The LECLs of >50% of tetraspanins include a Pro-x-x-Cys-Cys sequence (the PxxCC motif), in which 'x' is any amino acid. In addition, various other residues (mainly in the TM domains) are relatively well conserved among tetraspanins (Fig. 1). A combination of all the above features distinguishes tetraspanins from a diverse group of proteins that have four transmembrane domains, some of which [e.g. L6 antigen (Marken et al., 1992; Wright and Tomlinson, 1994), il-TSP (Wice and Gordon, 1995) and sarcospan (Crosbie et al., 1997)] were originally described as members of the tetraspanin superfamily.

Tetraspanins are implicated in a variety of normal and pathological processes, such as tissue differentiation (Boismenu et al., 1996), egg-sperm fusion (Le Naour et al., 2000; Miyado et al., 2000), tumor-cell metastasis (Boucheix et al., 2001) and virus-induced syncytium formation (Fukudome et al., 1992). Nevertheless, the biochemical function of the tetraspanin proteins remains undefined. No membrane or soluble protein has yet been shown to be a physiological receptor/ligand for tetraspanins [except for the hepatitis C virus envelope glycoprotein E2 (Flint et al., 1999)]. The N- and C-termini of tetraspanins, although well preserved across vertebrate species, exhibit no similarities between the individual family members. Thus, despite their relatively short lengths, these regions might have distinct functions. Computer-assisted analysis of the cytoplasmic domains of tetraspanins does not reveal any homology to well defined structural modules or motifs [except for the tyrosine-based sorting motif (see below)]. Given no obvious clues to their biochemical function, tetraspanins are predicted to represent transmembrane scaffolds that control the presentation and spatial organisation of various membrane complexes (Maecker et al., 1997).

Here, I discuss recent work concerning the structural and functional aspects of the complexes of tetraspanins with integrins.

*The author found in the GenBank database at least four new tetraspanin-like EST clones sequenced from high eukaryotes. The *Drosophila melanogaster* genome contains 37 tetraspanins (Todres et al., 2000).

†The LECLs of the tetraspanins rom-1 and RDS have an additional cysteine, which precedes the CCG-sequence (Bascom et al., 1992; Travis et al., 1989). The LECL of Tspan-5 has an additional pair of cysteines (Todd et al., 1998).

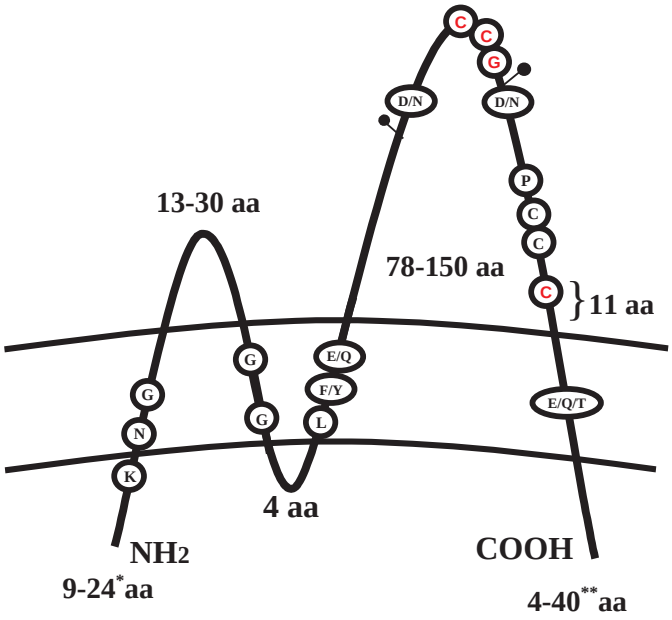

Fig. 1. Schematic representation of a generic tetraspanin. The CCG motif and the position of the last cysteine residue in the large extracellular loop are conserved in all tetraspanins. The PxxCC motif is found in >65% of tetraspanins. Encircled residues are found in > 60% of tetraspanins. Most tetraspanins (with the exception of CD81 and NET-2) have a consensus site for N-linked glycosylation in the large extracellular loop (LECL) (marked as). Note that a glycosylation site for CD9 was mapped to the small extracellular loop.
***** The N-terminal region of oculospanin contains 78 residues.
****** The C-terminal regions of RDS-1, ROM-1 and NET-2 contain 63, 65 and 63 residues, respectively.

The structural basis for integrin-tetraspanin complex assembly

Integrins are a large group of cell-surface αβ heterodimers that function as key adhesion receptors for the extracellular matrix (ECM). Since the first report describing the association of tetraspanin CD9 with αIIbβ3 integrin in stimulated platelets (Slupsky et al., 1989), various other integrin-tetraspanin protein complexes have been identified in many different cell types (Table 1). Although the association of certain integrins (e.g. α3β1, α4β1 and α6β1) with tetraspanins is observed in all cells in which the proteins are co-expressed, the others (e.g. α2β1, α5β1 and α6β4) form complexes with tetraspanins only in particular cell types*.

In spite of numerous reports describing the association of various tetraspanins with integrins, a detailed analysis of the integrin-tetraspanin interface is still lacking. A standard mapping approach involving either generation of chimeras or site-directed mutagenesis of tetraspanins is complicated by the fact that all tetraspanins that are expressed in a given cell can form complexes with each other and with at least one, but more often with two or three, different integrin heterodimers. Furthermore, in most cases it has been difficult to isolate single integrin-tetraspanin complexes. Thus, even if a specific mutation affects a direct contact between a tetraspanin and an integrin, the tetraspanin can still remain linked to the complex via an elaborate tetraspanin network [often referred to as the tetraspan web (Rubinstein et al., 1996)].

Although the association of most tetraspanins with integrins can be observed only in the presence of so-called 'mild' detergents, the CD151-α3β1, CD151-α6β1 and CD81-α4β1 complexes seem to be more stable given that they can withstand conditions (e.g. the presence of Triton X-100 and Digitonin) that disrupt all other integrin-tetraspanin and tetraspanin-tetraspanin interactions (Berditchevski et al., 1997a; Serru et al., 1999; Yauch et al., 1998). This suggests that the associations of CD151 and CD81 with their respective integrins are direct. Furthermore, by directly interacting with the integrins, these tetraspanins might bring other family members into integrin proximity. A recent report suggests that the tetraspanin Tspan-3 functions in a similar fashion, although a specific β1-integrin partner has not been identified (Tiwari-Woodruff et al., 2001).

The relative stability of the CD151-α3β1 complex has allowed delineation of the interacting regions of the α3 integrin subunit and tetraspanin. In CD151, the sequence between residues Leu149 and Glu213 within the second half of the LECL is necessary and sufficient to confer stable association with α3β1 integrin (Yauch et al., 2000; Berditchevski et al., 2001). This region includes all six cysteine residues present in the CD151 LECL, and mutation of the consecutive cysteine residues in the conserved CCG and PxxCC motifs precludes the direct association of CD151 with the integrin (Berditchevski et al., 2001). Hence, rather than being defined by a short linear sequence, the integrin-binding surface of CD151 is likely to be formed by various parts of the Leu149-Glu213 region, whose ternary fold is supported by the cysteine residues. It is not known whether this part of the CD151 LECL is also required for stabilisation of the CD151-α6β1 complex. The CD151 LECL is not required for the heterotypic CD151-tetraspanin interactions, however, and, therefore, cannot be responsible for bringing more distal tetraspanins to the CD151-α3β1 complex (Berditchevski et al., 2001).

Given the crucial role of the LECL in the CD151-α3β1 interaction, it is not surprising that the tetraspanin-binding site has been mapped to the extracellular portion of the α3 integrin subunit (Yauch et al., 1998; Yauch et al., 2000). Accordingly, substitution of the transmembrane and cytoplasmic domains of the α3 subunit with the corresponding regions of α5 does not affect its association with tetraspanins (Yauch et al., 1998). Using inter-integrin chimeras, Yauch and co-workers showed that part of the 'stalk' region within the α3 extracellular domain (the sequence between residues 569-705) is required for stable association with CD151 (Yauch et al., 2000). Although the interaction between α4β1 and the tetraspanin CD81 has not been examined in detail, an early study established that mutation of two metal-binding sites in the α4 integrin subunit diminished the stability of the complex (Mannion et al., 1996). Notably, corresponding mutations in α3 did not affect the association with CD151 (Yauch et al., 2000), which suggests that the interacting interfaces of the CD151-α3β1 and CD81-α4β1 complexes differ. Interestingly, homology modelling predicts that the α3β1-interacting part of CD151 and the corresponding region of CD81 have different folds (Seigneuret et al., 2001).

A conformational change in integrin receptors is an important mechanism for controlling their ligand-binding

*Some of the discrepancies observed in the literature may reflect differences in the purification conditions used in the immunoprecipitation experiments.

Table 1. Integrin-tetraspanin complexes

Tetraspanin	Integrin	Cells	References
CD9	α1β1	Epithelia (cervix)	Lozahic et al., 2000
	α2β1	Vascular smooth muscle, keratinocytes	Scherberich et al., 1998; Jones et al., 1996
	α3β1	Epithelia (skin, breast, endometrium, colon and kidney), trophoblasts, endothelia, Schwann cells, vascular smooth muscle, fibrosarcoma, melanoma	Scherberich et al., 1998; Jones et al., 1996; Berditchevski et al., 1996; Hadjiargyrou, et al., 1996; Yáñez-Mó et al., 1998; Yáñez-Mó et al., 2001; Hirano et al., 1999; Nakamura et al., 1995; Park et al., 2000; Serru et al., 1999
	α4β1	B cells, T cells	Rubinstein et al., 1994
	α5β1	B cells, myocytes, trophoblasts	Rubinstein et al., 1994; Hirano et al., 1999; Tachibana and Hemler, 1999
	α6β1	Epithelia (breast, endometrium), trophoblasts, fibrosarcoma	Berditchevski et al., 1996; Hirano et al., 1999; Park et al., 2000
	α7β1	Myocytes	Tachibana and Hemler, 1999
	α6β4	Keratinocytes	Jones et al., 1996
	αIIbβ3	Platelets	Slupsky et al., 1989
CD53	α4β1	T cells	Mannion et al., 1996
CD63	α3β1	Epithelial (breast), fibrosarcoma	Berditchevski et al., 1996
	α4β1	T cells	Mannion et al., 1996
	α6β1	Epithelial (breast), fibrosarcoma	Berditchevski et al., 1996
	αMβ2	Neutrophils	Skubitz, et al., 1996
	αIIbβ3	Platelets	Israels et al., 2001
CD81	α3β1	Epithelia (breast, cervix), fibrosarcoma, neurites, myocytes	Berditchevski et al., 1996; Serru et al., 1999; Tachibana and Hemler, 1999; Stipp and Hemler, 2000
	α4β1	T cells, B cells, erythroleukaemia, myocytes	Tachibana and Hemler, 1999; Mannion et al., 1996
	α5β1	Myocytes	Tachibana and Hemler, 1999
	α6β1	Epithelia (breast), fibrosarcoma, Rhabdomyosarcoma	Berditchevski et al., 1996; Mannion et al., 1996
	α7β1	Myocytes	Tachibana and Hemler, 1999
CD82	α3β1	Epithelia (breast)	Berditchevski and Odintsova, 1999
	α4β1	T cells, B cells, rhabdomyosarcoma	Mannion et al., 1996
	α5β1	Hamster ovary	Ono et al., 2000
	α6β1	Rhabdomyosarcoma	Mannion et al., 1996
CD151	α3β1	Epithelia (skin, breast, cervix, kidney, colon), endothelia, neurites, fibrosarcoma	Yáñez-Mó et al., 1998; Yáñez-Mó et al., 2001; Serru et al., 1999; Stipp and Hemler, 2000; Berditchevski and Odintsova, 1999; Yauch et al., 1998; Sterk et al., 2000
	α4β1	Megakaryocytes, erythroleukaemia	Fitter et al., 1999
	α5β1	Megakaryocytes, T cells, erythroleukaemia, endothelia	Fitter et al., 1999; Sincock et al., 1999; Hasegawa et al., 1998
	α6β1	Epithelia (cervix, colon), B-cells, fibrosarcoma, megakaryocytes, endothelia, erythroleukaemia	Serru et al., 1999; Yauch et al., 1998; Fitter et al., 1999; Sincock et al., 1999
	α6β4	Keratinocytes, endothelia	Sterk et al., 2000; Sincock et al., 1999
	αIIββ3	Erythroleukaemia	Fitter et al., 1999
Nag2/Tspan4	α3β1	Epithelia (breast), fibrosarcoma	Tachibana et al., 1997
	α6β1	Epithelia (breast), fibrosarcoma	Tachibana et al., 1997
Tspan3	αβ1	Oligodendrocytes	Tiwari-Woodruff et al., 2001
CO-029	α3β1	Epithelia (colon, pancreatic)	Serru et al., 1999; Claas et al., 1998
	α6β1	Epithelia (colon, pancreatic)	Serru et al., 1999; Claas et al., 1998

Only original reports describing a particular integrin-tetraspanin association in a particular cell type are cited.

activity (Humphries, 2000; Plow et al., 2000). As yet, there is no evidence demonstrating that their association with tetraspanins influences integrin conformation. Neither is there any indication that a particular conformational state favours the association of integrins with tetraspanins. The results of numerous studies indicate that various integrin-tetraspanin complexes can be immunoprecipitated equally well regardless of whether function-blocking, neutral or function-activating anti-integrin antibodies are used. Divalent cations, which activate (or inhibit) the ligand-binding function of integrins (e.g. Mg^{2+}, Mn^{2+} and Ca^{2+}), have no effect on integrin association with tetraspanins (Longhurst et al., 1999; Mannion et al., 1996; Yáñez-Mó et al., 2001a). Finally, ligand binding does not seem to influence the stability of various integrin-tetraspanin complexes (Israels et al., 2001; Longhurst et al., 1999; Yáñez-Mó et al., 2001a).

An interesting aspect of the integrin-tetraspanin complex formation has been described recently: Ono and co-workers

found that the association of CD82 with α3β1 and α5β1 depends on the glycosylation state of both tetraspanin and integrins (Ono et al., 2000). Given that the degree of CD82 glycosylation varies in different cell types (White et al., 1998), this may represent a tissue-specific control mechanism that regulates the assembly of the CD82-α3β1 and CD82-α5β1 complexes.

The role of tetraspanins in integrin-mediated cell adhesion and migration

Both antibody-interference experiments and comparative analysis of cell adhesion have shown that in most cell types the contribution of tetraspanins to β1-integrin-mediated cell adhesion or spreading on the ECM proteins is insignificant (Sugiura and Berditchevski, 1999; Berditchevski et al., 1996; Claas et al., 1998; Fitter et al., 1999; Shaw et al., 1995; Stipp and Hemler, 2000). This is not surprising given that there is no evidence that the association with tetraspanins can directly affect integrin affinity for ECM ligands, integrin clustering or linkage of integrins to the actin cytoskeleton, all of which are key factors in establishing strong adhesive contacts with ECM substrates. In addition, in most cells, tetraspanins are absent from focal contacts (see below) – the complex adhesion structures primarily responsible for strong attachment to the ECM.

In contrast, numerous reports describe the involvement of tetraspanins in both homotypic and heterotypic cell-cell adhesion (Barrett et al., 1991; Bradbury et al., 1992; Cao et al., 1997; Fitter et al., 1999; Lazo et al., 1997; Lagaudriere-Gesbert et al., 1997; Letarte et al., 1993; Masellis-Smith et al., 1990; Schick and Levy, 1993; Shibagaki et al., 1998; Skubitz et al., 1996; Toothill et al., 1990). The possible involvement of tetraspanins in intercellular adhesion has been a focus of a recent review (Yáñez-Mó et al., 2001b). It has to be emphasised that intercellular contacts are controlled by a diverse range of receptor-ligand interactions in which integrins play either a major (as in hematopoetic cells) or an auxiliary role. Given that tetraspanins can form complexes with various other transmembrane proteins, their role in regulating intercellular contacts may also involve non-integrin adhesion receptors (Lazo et al., 1997).

The involvement of tetraspanins in cell motility is well documented. Numerous reports published over the past ten years have demonstrated that tetraspanins are implicated in migration of monolayers (e.g. in wound closure), as well as in the random and chemotactic motility of various cell types (Ikeyama et al., 1993; Klein-Soyer et al., 2000; Miyake et al., 1991; Ono et al., 2000; Penas et al., 2000; Radford et al., 1997; Shaw et al., 1995; Sincock et al., 1999; Yáñez-Mó et al., 1998; Yáñez-Mó et al., 2001a). In addition, recent data indicate that tetraspanins are involved in more complex biological phenomena driven by integrin-ECM interactions. These include invasive migration of carcinoma cells within the 3D ECM (Sugiura and Berditchevski, 1999), collagen-gel contraction (Scherberich et al., 1998), morphogenetic re-organisation of monolayers of epithelial cells (Yáñez-Mó et al., 2001a) and neurite outgrowth (Stipp and Hemler, 2000).

Cell migration is a complex phenomenon that is controlled by highly regulated processes both at the leading edge and at the rear of the cell (Lauffenburger and Horwitz, 1996). In both stationary and migratory cells, tetraspanin-containing protein complexes are highly abundant at the outermost cell periphery (Berditchevski and Odintsova, 1999; Berditchevski et al., 1997b). These highly dynamic parts of the cell are engaged in transient interactions with the substrate and trigger the initial set of biochemical signals that leads to the assembly of more stable attachment structures (e.g. focal adhesions). Two recent reports indicate that tetraspanin-containing adhesion complexes may control the protrusion activity in migrating cells. Firstly, antibody-interference experiments have demonstrated that in keratinocytes, tetraspanins regulate lamellipodia formation (Baudoux et al., 2000). Secondly, elongation of the invasive protrusions of breast carcinoma cells embedded into the 3D ECM can be stimulated by antibodies to various tetraspanins and the α3 integrin subunit (Sugiura and Berditchevski, 1999). Detailed time-lapse analysis has shown that antibody treatment attenuates retraction of the extending protrusions (Sugiura and Berditchevski, 1999).

Current data suggest that the pro- or anti-migratory activities of tetraspanins are not limited to a particular ECM ligand (Shaw et al., 1995, Domanico et al., 1997). Furthermore, binding of the tetraspanins can affect integrin-mediated cell migration in both a ligand-dependent and a ligand-independent fashion (Domanico et al., 1997). In most cells, the tetraspanin-containing peripheral adhesion complexes are devoid of cytoskeletal and signalling components typically found in more stable adhesion structures, such as focal adhesions and Rac-dependent focal complexes (e.g. vinculin, paxilin and FAK) (Berditchevski and Odintsova, 1999; Penas et al., 2000). In contrast, tetraspanins are colocalised with MARCKS (myristoylated alanine-rich C kinase substrate), one of the prominent substrates for different members of the protein kinase C (PKC) family (Berditchevski and Odintsova, 1999). One of the proposed functions of MARCKS is regulation of cortical cytoskeleton dynamics during cell spreading and migration (Wiederkehr et al., 1997). Given that certain tetraspanins can associate with PKC enzymes (Zhang, et al., 2001a), it is conceivable that the tetraspanin-containing adhesion complexes affect the actin-reorganising activity of MARCKS.

In sections of normal skin and cultured keratinocytes, the anti-CD151 antibody (but not several other anti-tetraspanin antibodies) label hemidesmosomes – specialised junctional complexes that mediate stable attachment of cells to the basement membrane (Sterk et al., 2000). Thus, CD151 might have opposing functions in cell migration: in non-transformed epithelial cells, it could be involved in the stabilization of cell attachment, but in carcinomas, the elevated expression of CD151 potentiates their pro-migratory phenotype (Testa et al., 1999).

Finally, in some cells (epithelia and endothelia), integrin-tetraspanin complexes are enriched at cell-cell contact sites (Nakamura et al., 1995; Penas et al., 2000; Yáñez-Mó et al., 2001b), where they may affect migration indirectly by regulating the dynamics of intercellular communication.

Signal transduction by integrin-tetraspanin protein complexes

Several recent studies have provided evidence that tetraspanins are involved in adhesion-dependent signalling mediated by integrins. In Raji cells (a B cell line), ectopically expressed

CD9 associates with α4β1 and α6β1 integrins and potentiates fibronectin- and laminin-1-dependent tyrosine phosphorylation of 130 kDa and 69 kDa proteins (Shaw et al., 1995). In addition, expression of CD9 in fibrosarcoma cells specifically affects the de-phosphorylation rate of FAK (Berditchevski and Odintsova, 1999), and clustering of the α3β1-tetraspanin complexes in breast carcinoma cells stimulates the PI3-kinase-dependent signalling pathway (Sugiura and Berditchevski, 1999).

The contribution of tetraspanins to adhesion-dependent signalling might be linked with their ability to recruit certain signalling enzymes into the integrin complexes (Hemler, 1998). A number of tetraspanins, including CD9, CD63, CD81, A15/Talla-1 and CD151 (but not CD82, CD37, CD53 or NAG-2/Tspan-4), are associated with type II phosphatidylinositol 4-kinase (PtdIns 4-K), one of the key enzymes in synthesis of C-4 phosphoinositides (Berditchevski et al., 1997b; Yauch and Hemler, 2000). Furthermore, the association with CD151 seems to be critical for tethering of PtdIns 4-K to α3β1 integrin (Yauch and Hemler, 2000). Given the lack of apparent similarity between the cytoplasmic portions of the PtdIns 4-K-linked tetraspanins, the association is probably mediated by another part(s) of the protein. Although the interaction of PtdIns 4-K with tetraspanins does not require their association with integrins, not all tetraspanin-linked integrins (e.g. α4β1 and α6β1) are associated with the PtdIns 4-K activity (Yauch and Hemler, 2000). This suggests that additional mechanisms (or protein components) that control the association of the enzyme with integrins exist. Whether or not binding of the α3β1 integrin to ECM ligands regulates the activity of the enzyme within the complex is not known.

Treatment of cells with phorbol ester (PMA) induces association of various tetraspanins (e.g. CD9, CD53, CD81, CD82 and CD151) with two members of the PKC family, α and βII (Zhang et al., 2001a). The specificity of the tetraspanin-PKC interaction is further strengthened by the fact that other PKC enzymes, including PKCε, PKCζ and PKCμ, are not associated with the tetraspanin complexes (Zhang et al., 2001a). An interaction with integrins is not required for the association of tetraspanins with PKC, which suggests that tetraspanins play a critical role in recruiting PKC into the integrin complexes (α3β1 and α6β1). Furthermore, PKC-dependent phosphorylation of the α3 integrin subunit is critical for cell migration and for the adhesion-dependent signalling mediated by α3β1 (Zhang et al., 2001b).

Two Src family tyrosine kinases, Lyn and Hck, and unspecified serine/threonine kinase activities, are associated with the β2-integrin–CD63 complex and might play an important role in the CD63-induced upregulation and activation of β2 integrins in human neutrophils (Skubitz et al., 1996).

The ability to associate simultaneously with one another and various classes of transmembrane proteins might mean that tetraspanins can transmit lateral signals between integrins and other surface receptors. This could further diversify the contribution of tetraspanin proteins to adhesion-dependent signalling. For example, in B cells, the network of tetraspanins may juxtapose α4β1 and α5β1 integrins with the CD21-CD19-Leu13 complex, so that antibody-induced crosslinking of integrins or tetraspanins induces phosphorylation of CD19 (Horvath et al., 1998; Xiao et al., 1996). Conversely, clustering of CD19 complexes induces tyrosine-kinase-dependent adhesion of B cells to cell-deposited fibronectin, a process mediated by α4β1 integrin (Behr and Schriever, 1995).

Table 2. A tyrosine-based internalisation motif (Yxxϕ) found at the C-termini of tetraspanins

CD151	...*YRSL*KLEHY
CD63	...KSIRSG*YEVM*
A15	SRFITANQ*YEMV*
CO-029	...*YCQI*GNK
CD37	...RNLDHV*YNRL*ARYR
CD82	...RHVHSED*YSKV*PKY
TSP-1	...YL*YCNL*Q
TSP-3	...CRRSRDPA*YELL*ITGGTYA
TSP-6	...SRAITNNQ*YEIV*
UP1a	...YF*YTML*

The role of tetraspanins in integrin maturation and trafficking

Biosynthetic labelling experiments indicate that there is a clear hierarchy in assembly of various α3β1-integrin–tetraspanin complexes: the interaction with CD151 takes place early during integrin biosynthesis – the association of CD151 with the pre-α3 subunit is detectable even before the β1 subunit joins the complex (Berditchevski et al., 2001). Furthermore, CD151 mutants that are sequestered in the endoplasmic reticulum (ER) retain their ability to associate with α3β1 (Berditchevski et al., 2001). In contrast, other tetraspanins, including CD9, CD63 and CD81, are associated only with the mature heterodimer. In cells that have an abundant pool of intracellular integrins, CD9 (but not CD81 or CD82) is associated with the pre-β1 subunit and calnexin, an ER chaperone protein (Rubinstein et al., 1997). Whether or not these early associations with tetraspanins are required for proper biogenesis of the integrin heterodimers remains to be determined.

Immunoelectron microscopy and immunofluorescence studies have demonstrated that tetraspanins are abundant on various types of intracellular vesicles (Escola et al., 1998; Hamamoto et al., 1994; Hotchin et al., 1995; Peters et al., 1991; Sincock et al., 1999). These data point to a possible role for tetraspanins in turnover and/or sorting of integrins. Several tetraspanins contain a tyrosine-based sorting motif (Tyr-X-X-φ) at their C-termini (Table 2) that might recruit clathrin adapter proteins to the integrin complexes and thereby direct them along various trafficking routes (Bonifacino and Dell'Angelica, 1999). The tetraspanin-associated PtdIns 4-K and PKC can also contribute to this 'sorting' function of tetraspanins (Fig. 2). Indeed, activation of PKCα in mammary epithelial cells facilitates internalisation of β1 integrins (Ng et al., 1999).

Integrin-tetraspanin complexes and lipid rafts

Compartmentalisation of various types of surface receptors into specialised membrane microdomains can dramatically affect their signalling capacity (Schlegel et al., 1999; Xavier and Seed, 1999). One type of surface compartment, the glycosphingolipid-cholesterol-enriched microdomains [GEM; also referred to as lipid rafts (Kurzchalia and Parton, 1999)], has attracted a great deal of attention as a place where

signalling events associated with activation of various transmembrane receptors are generated (Simons and Toomre, 2000). Although several studies have examined membrane compartmentalisation of tetraspanins and integrins, the relationship between the tetraspanin web and the lipid rafts remains controversial. An early report demonstrated that certain amounts of CD9 and αIIbβ3 integrin are co-fractionated with the protein components of lipid rafts in low-density fractions in a sucrose gradient (Dorahy et al., 1996). More recently, the same has been shown to occur for other tetraspanins (e.g. CD9, CD63, CD81, CD82 and CD151) and integrins (α3β1 and αLβ2) (Claas et al., 2001; Odintsova et al., 2000; Skubitz et al., 2000). Furthermore, tetraspanins are partially colocalised with the ganglioside GM1, a marker for lipid rafts (Claas et al., 2001). However, both biochemical (sucrose gradient fractionation) and immunofluorescence (colocalisation with GPI-linked proteins) experiments clearly indicate that a significant proportion of the integrin-tetraspanin protein complexes reside outside lipid rafts (Berditchevski et al., 1996; Claas et al., 2001; Dorahy et al., 1996). Thus, it has been proposed that the tetraspanin-enriched membrane patches may represent a new class of membrane microdomain that differs from conventional rafts (Claas et al., 2001). Furthermore, a non-uniform distribution could reflect either structural and, possibly, functional heterogeneity of the complexes or their lateral dynamics in the plasma membrane. It is also possible that integrin-tetraspanin complexes shuttle in and out of rafts and thereby control the recruitment of other proteins into these compartments (Claas et al., 2001). This function may be facilitated by fatty acyl groups that are covalently linked to tetraspanins (Seehafer et al., 1988; Seehafer et al., 1990).

Perspectives

With various integrin-tetraspanin combinations being described in different cell types, the main challenge remains for us to understand the structural basis of all these interactions. In particular, it will be important to establish whether the regions that are engaged in the α3β1-CD151 interaction also form the contact interface of other integrin-tetraspanin pairs. Furthermore, what is the hierarchical order of the interactions between integrin-proximal CD151-CD81 and other tetraspanins? A detailed pair-wise analysis of various tetraspanin-tetraspanin interactions should determine the spatial organisation of integrin-tetraspanin clusters and set up a structural framework for future functional analyses. An important aspect of this work will be to establish which part(s) of tetraspanins controls their association with PtdIns 4-K and PKC isoforms. Current data pose a number of intriguing questions for the future. Firstly, why is PtdIns 4-K-associated activity not detected in the α6β1-tetraspanin and α4β1-tetraspanin complexes (Yauch et al., 1998)? Are there integrin-specific exclusion/inhibitory mechanisms? A comprehensive biochemical dissection of integrin-tetraspanin complexes and extensive mutagenesis of their components should answer these questions. Furthermore, what are the physiological pathways that regulate the association of PtdIns 4-K and PKC with the complexes and their activities? Early reports indicated that the activity of PtdIns-K is upregulated in cells treated with EGF (Kauffmann-Zeh et al., 1994). Does EGF also affect the activity of the enzyme associated with the integrin-tetraspanin complex? Do tetraspanins, which are known to associate with the EGF receptor (Odintsova et al., 2000), facilitate this process? Many physiological stimuli are known to activate PKC, which, in turn, may affect both adhesive and non-adhesive properties of integrins, including their clustering, post-ligand binding signalling, and turnover. It will be important to identify those stimuli that are specifically directed towards the isoforms of PKC associated with tetraspanin complexes. Such detailed dissection of both lateral and vertical 'signalling waves' involving tetraspanins should answer an important outstanding question – whether tetraspanins function as a combined signalling entity (e.g. as components of the 'tetraspanin web') or whether each of the integrin-tetraspanin complexes makes its own specific contribution to adhesion-dependent signalling.

The function of tetraspanins is clearly important in integrin-driven cell migration. Given the distinct composition of integrin-tetraspanin adhesion complexes, it will be crucial to identify specific proximal targets for the activated integrin-tetraspanin complexes. Tetraspanin-associated PtdIns 4-K can enhance synthesis of phosphoinositides in proximity to integrins (Fig. 2). This may affect the actin-binding properties of a number of integrin-associated cytoskeletal proteins (e.g. α-actinin, talin and filamin). Alternatively, locally generated phosphoinositides may function as anchors for the recruitment

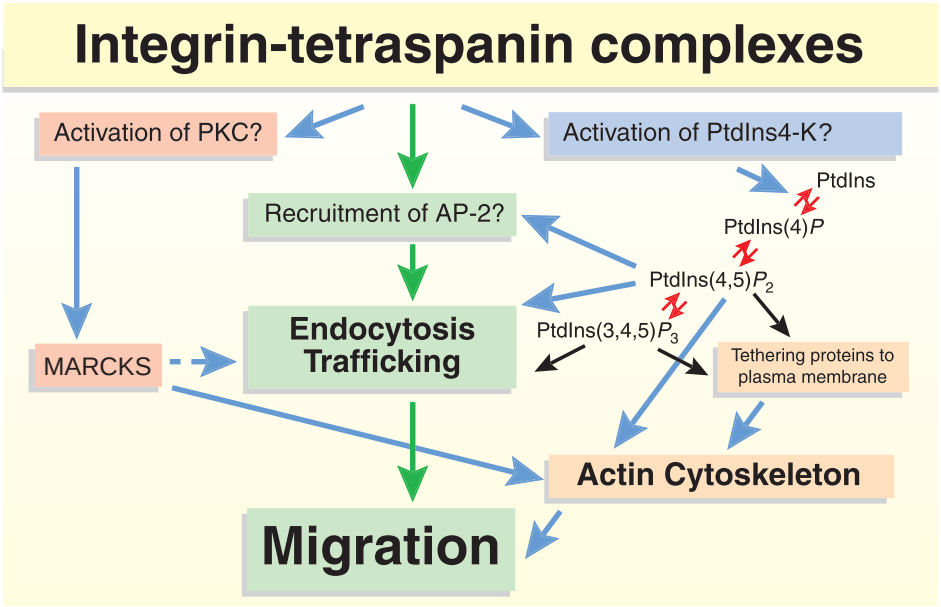

Fig. 2. A hypothetical model showing how integrin-tetraspanin complexes may influence cell migration.

of cytoplasmic proteins (e.g. MARCKS) into the integrin vicinity (Fig. 2). Similarly, the activity of the tetraspanin-associated PKC may be directed towards various protein targets (both cytoskeletal and signalling). Identification of new components within integrin-tetraspanin complexes and studying their involvement in PKC-dependent signalling should become a focus of future experiments. Another important aspect of future work will be to examine the spatial dynamics of the complexes in migrating cells, particularly in relation to cytoskeleton and adhesion-related signalling proteins. These experiments should place tetraspanin-dependent processes into a specific signalling context and answer the question of whether there is a functional link between tetraspanin-containing adhesion complexes and the more stable focal adhesions.

It is currently recognised that targeted delivery of integrins to the leading edge of migrating cells and their recycling may help to perpetuate lamellipodial extensions (Fabbri et al., 1999; Lauffenburger and Horwitz, 1996; Pierini et al., 2000). Certainly, the occurrence of the tyrosine-based sorting signal in some tetraspanins equips them with the potential to serve as navigators that direct integrin trafficking during the migration. A comparative real-time analysis of integrin trafficking in cells expressing tetraspanins in which the sorting signal is obliterated should establish whether this potential is indeed realised.

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