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Biochemical and Biophysical Research Communications 304 (2003) 107–112
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Multiple levels of interactions within the tetraspanin web☆

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Received 24 February 2003

Abstract

The tetraspanin web refers to a network of molecular interactions involving tetraspanins and other molecules. Inside the tetraspanin web, small primary complexes containing only one tetraspanin and one specific partner molecule such as CD151/α3β1 integrin and CD9/CD9P-1 (FPRP) can be observed under particular conditions. Here we demonstrate that when cells are lysed with Brij97, the tetraspanins CD151 and CD9 allow and/or stabilize the interaction of their partner molecules with other tetraspanins and that their two partners associate under conditions maintaining tetraspanin/tetraspanin interactions. The tetraspanins were also found to partition into a detergent-resistant membrane environment to which the integrin α3β1 was relocalized upon expression of CD151.

Keywords: Tetraspanin; CD9; CD151; Integrin; Interaction; Detergent-resistant membrane

The tetraspanins are four transmembrane region molecules found in all cells but erythrocytes. They have been implicated in many cellular functions such as adhesion, migration, co-stimulation, signal transduction, and sperm–egg fusion [1–4]. Increasing evidences also show the importance of tetraspanins in pathological situations including certain cases of X-linked mental retardation [5], hepatitis C [6], malaria [7], and metastasis [8,9] (for review see [10]).

How tetraspanins function at the molecular level is poorly understood. A unique property of these molecules is their ability to associate with many other cell surface molecules. Remarkably, in mild lysis conditions (using detergents like Brij or CHAPS), they clearly co-immunoprecipitate the same molecules [11–13], including β1 integrins in most cell types (in particular α4β1, α3β1, and α6β1), CD4, CD8, MHC class II molecules or CD19 in lymphoid cells (for review [1,14]). Intracellular signaling enzymes such as phosphatidylinositol 4-kinase and protein kinase C may also associate with several tetraspanins [15,16]. These data, together with the strong association of tetraspanins with each other, show that they are involved in a network of molecular interactions, the "tetraspanin web" [12]. Monoclonal antibodies (mAbs) to different tetraspanins often produce similar effects in vitro and this suggests that mAbs similarly engage the tetraspanin web in a way that could modulate the activity of the non-tetraspanin molecules. For example, modulation of migration produced by anti-tetraspanin mAb is proposed to be related to the association with integrins [17–22] while tyrosine phosphorylation observed in lymphoid B cells following antibody-ligation of tetraspanins has been suggested to be to some extent a consequence of their association with CD19, which is itself phosphorylated under such stimulation [23,24].

Further characterization of the tetraspanin web is essential to understand how tetraspanins work. Two steps have already been taken in this direction. First, it

☆ *Abbreviations:* MβCD, methyl-β-cyclodextrin; DRM, detergent-resistant membrane.

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0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00545-X

has been shown that under particular lysis conditions, primary complexes containing only one tetraspanin associated with a limited number of molecular partners could be observed. If the interaction of CD151 with the integrin α3β1 could be observed in the presence of Triton X-100 [25], many more primary complexes were identified when cells were lysed with digitonin. These include CD81/CD19, CD81/α4β1, CD151/α6β1, CD9/CD9P-1 (FPRP), CD81/CD9P-1, CD9/EWI-2, and CD81/EWI-2 [24,26–30]. The second important step has been the demonstration that the molecules present in one primary complex interacted directly with each other. This was achieved using chemical cross-linking experiments, for the CD151/α3β1 and the CD9/CD9P-1 pairs. These experiments also demonstrated the limited size of these primary complexes [29,31,32]. These data revealed some preferences of interactions within the tetraspanin web, which could be explained by either different stabilities of tetraspanin/non-tetraspanin interactions or by different levels of interactions. In the present study, we investigated whether the expression of a particular tetraspanin was required for the interaction of its molecular partner with the other tetraspanins, using the CD151/α3β1 and CD9/CD9P-1 pairs as a model, because of the stability and the high stoichiometry of these complexes. Also, the tetraspanins were shown to partition in part in a detergent-resistant membrane (DRM) environment with properties different from those of the plasma membrane microdomains enriched in cholesterol, glycosphingolipids, and GPI-anchored molecules known as rafts [33,34]. CD151 was demonstrated to induce the redistribution of α3β1 integrin within this environment.

Materials and methods

Monoclonal antibodies. Anti-tetraspanins mAbs used in this study were SYB-1, ALB-6 (CD9, [12]), TS81 and Z81 (CD81, [29,35]), TS151 and TS151r (CD151, [28,29]), TS53 (CD53, IgG1), and TS82 (CD82, [29]). mAb 1F11 is directed to CD9P-1 [29] mAbs to integrins used for this study were C9 (β1) [12], M-KID 2 (α3 [36]), V5-vjf (α5) [28], and Gi9 (α2 Coulter-Immunotech, Marseille, France), and rabbit polyclonal antibodies to α3 and α5 integrins were purchased from Chemicon (Temecula, CA). A goat polyclonal antibody to CD71 was from Santa-Cruz biotechnology (Santa-Cruz, CA).

Cell lines, cell culture, and transfection. The lymphoid B cell line Daudi was cultured in RPMI 1640 medium (Eurobio, Les Ulis, France) supplemented with 10% FCS, 2 mM glutamine, and antibiotics (all from Life Technologies, Cergy-Pontoise, France). The adherent cell lines HeLa (cervical carcinoma), A431 (epidermoid), and CHO (Chinese Hamster Ovary) were grown in DMEM (Eurobio). CHO cells stably expressing CD9P-1 and CD9-transfected Daudi cells have previously been described [24,29]. For this study, Daudi cells were first transfected as previously described [24] with cDNA encoding α3 and β1 subunits (in pcDNA3, Invitrogen), and then with either CD151 or CD9 cDNA (in pcDNA3hygro, Invitrogen, Carlsbad, CA).

Cell lysis and immunoprecipitations. Adherent cells were lysed directly in the tissue culture flask (2 ml for a 75 cm^2 flask) and hematopoietic cells were lysed at the concentration of 2×10^7/ml, in a lysis buffer containing 1% detergent (Brij97, or digitonin, both from Sigma), 10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.02% NaN_3, 1 mM PMSF, 0.5 μg/ml leupeptin, 1 μg/ml pepstatin A, and 10 KIU/ml aprotinin. Digitonin was first dissolved in methanol at the concentration of 10% (w/v) and then diluted in lysis buffer without divalent cations. The samples were cleared by centrifugation at 12,000*g* and the supernatant was precleared overnight by addition 1/200 volume heat inactivated goat serum and 1/40th volume protein G–Sepharose beads (Amersham–Pharmacia, Rainham, UK) and an additional centrifugation at 12,000*g* for 15 min. Proteins were then immunoprecipitated by adding 1 μl ascitic fluid and 10 μl protein G–Sepharose beads to 200–400 μl of the lysate. After 5 h incubation at 4 °C under constant agitation, the beads were washed 5 times in lysis buffer. The immunoprecipitates were separated by 5–15% SDS–polyacrylamide gel electrophoresis under non-reducing conditions and transferred to a PVDF membrane (Amersham). Western blotting on immunoprecipitates was performed using biotinylated mAbs and a streptavidin-biotinylated horseradish peroxidase complex (Amersham), which was revealed by enhanced chemiluminescence (NEN, Boston, MA).

Equilibrium density gradient centrifugation. The cells were pelleted and lysed in an equal volume of 2× ice-cold lysis buffer containing 25 mM Tris–HCl, pH 7.5; 150 mM NaCl; 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.2 mM Na_3VO_4, protease inhibitors, and either 1% Brij97 or 0.5% Triton X-100. The ratio of lysis buffer volume to cell number was kept constant throughout the experiments. After a 30 min incubation on ice, the preparation was made 40% with respect to sucrose, in the lysis buffer without detergent. Then, 0.8 ml of lysate–sucrose mixture was sequentially overlaid with 2 ml of 30% sucrose and 1 ml of 4% sucrose prepared in the same buffer, without detergent, and the mixture was centrifuged at 200,000*g* for 14–16 h in an SW50.1 rotor (Beckman). The gradient was fractionated in 0.5-ml fractions from the top of the tube. All steps were at 4 °C except when indicated.

Results and discussion

CD151 and CD9 allow the association of their partners (integrin α3β1 and CD9P-1) with other tetraspanins

We first investigated whether the integrin α3β1 could associate with other tetraspanins in cells lacking CD151. Because we failed to identify a cell line expressing the integrin α3β1 but not CD151, experiments were performed using the B lymphoid cell line Daudi that lacks both CD151 and CD9. The integrin α3β1 was transfected in Daudi cells (Fig. 1A), and its possible association with the endogenous tetraspanins CD81 and CD53 was studied by co-immunoprecipitation after Brij97 lysis, a condition preserving tetraspanin/tetraspanin interactions. As shown in Fig. 1B, in these cells, the integrin α3β1 could not be detected in the CD81 and CD53 immunoprecipitates, and reciprocally, failed to co-immunoprecipitate CD81. This shows that the stable interaction of α3β1 integrin with tetraspanins other than CD151 requires an additional molecule. To determine whether this additional molecule was CD151, the α3β1-expressing Daudi cells were further transfected with CD151, or CD9 as a control. Their expression level was comparable to that of endogenous tetraspanins (CD81 and CD53) and to their expression level in other human

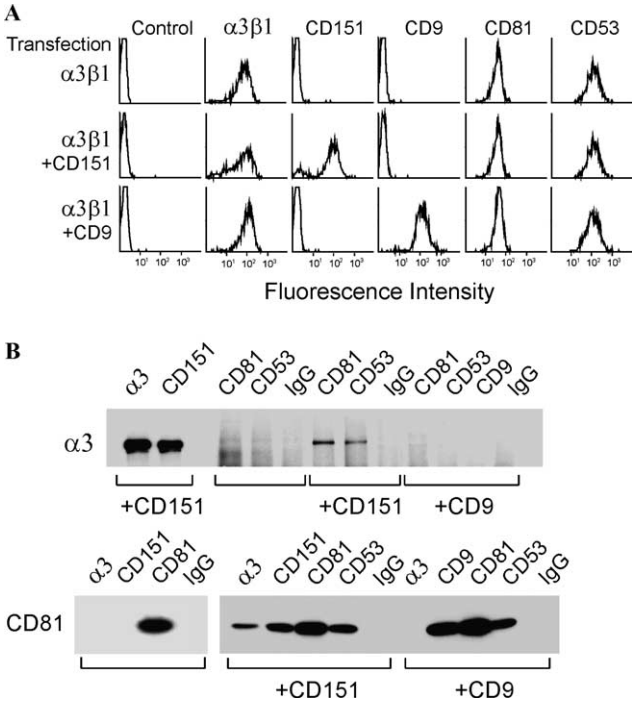

Fig. 1. CD151 allows the interaction of α3β1 integrin with other tetraspanins. (A) Flow-cytometric analysis Daudi cells transfected with α3β1 integrin alone or with CD151 or CD9. (B) Analysis of α3β1 integrin association with tetraspanins. α3β1 integrin-transfected Daudi cells, expressing or not CD9 or CD151, were lysed with Brij97 and immunoprecipitations were performed as indicated on the top of each lane. The blots were revealed with either a polyclonal mAb to α3 integrin (upper) or a biotin-labeled mAb to CD81 (Z81, lower).

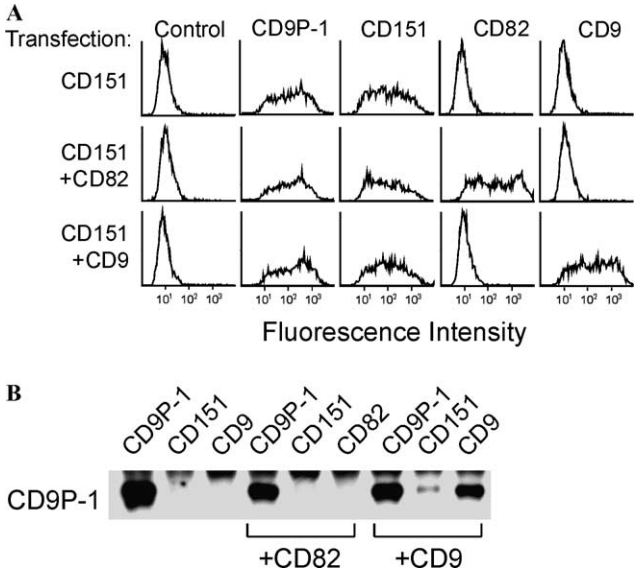

Fig. 2. CD9 specifically allows the interaction of CD9P-1 with CD151 and CD82. (A) Flow cytometric characterization of CHO cells stably expressing CD9P-1 and transiently transfected with CD151 alone or with CD9 or CD82. (B) Association of CD9P-1 with tetraspanins. CD9P-1-expressing CHO cells transiently transfected with CD9 or CD82 and CD151 were lysed with Brij97 and immunoprecipitations were performed as indicated on the top of each lane. The immunoprecipitates were analyzed by Western blotting using a biotin-labeled anti-CD9P-1 mAb 1F11.

cells (Fig. 1A, [28]). As expected, the co-expression of CD151 resulted in its strong association with the integrin α3β1 as shown by co-immunoprecipitation (Fig. 1B). Furthermore, the reactivity with the mAb TS151r, which only binds to CD151 when it is not complexed with α3β1 or α6β1 [28], indicated that ≈60% of surface CD151 was associated with α3β1 (data not shown). In the cell line expressing CD151, the integrin α3β1 was co-immunoprecipitated with both CD81 and CD53 while accordingly, CD81 appeared in the α3β1 immunoprecipitate (Fig. 1B). No association of α3β1 integrin with tetraspanins could be detected in cells expressing CD9 instead of CD151.

This result was extended to another tetraspanin/partner pair. CD9P-1 is a partner for both CD9 and CD81 [28,29]. Because we did not identify a human cell line not expressing these two tetraspanins, we used transfected CHO cells. CHO cells stably expressing CD9P-1 were transiently transfected with CD151 alone, or also with CD9 or CD82 (Fig. 2A), and the association of CD9P-1 with CD151 was studied by co-immunoprecipitation, after cell lysis in Brij97. CHO cells express endogenous hamster CD9 and CD81, and thus a basal level of interaction of CD9P-1 with CD151 was expected in the absence of ectopically expressed CD9. However, using cells expressing high levels of CD9P-1, this basal level was low and detectable only with long exposures, probably because most of endogenous CD9 or CD81 are engaged in other interactions. CD9, but not CD82, strongly associated with CD9P-1 and increased the association of CD151 with CD9P-1 (Fig. 2B).

Altogether, these data show that a tetraspanin induces or stabilizes the association of its molecular partner(s) with other tetraspanins. These data also strongly suggest that a tetraspanin interacts only *indirectly* with the molecular partners of other tetraspanins. In this regard, cross-linking experiment on cells expressing high levels of CD9 and CD151 did not reveal any CD151/CD9P-1 interaction under conditions in which CD9/CD9P-1 complexes were readily detected [29].

The integrin α3β1 associates with CD9P-1 under conditions preserving tetraspanin/tetraspanin interactions

We then investigated whether the β1 integrin and CD9P-1 could be present in the same complex. As shown in Fig. 3, a complex containing these two molecules could be detected when A431 cells were lysed in Brij97 to preserve the tetraspanin/tetraspanin interactions, but not when they were lysed in digitonin. Moreover, CD9P-1 associated with the integrin α3β1, a tetraspanin-associated integrin, but not with α2β1 or α5β1, two integrins not associated with tetraspanins.

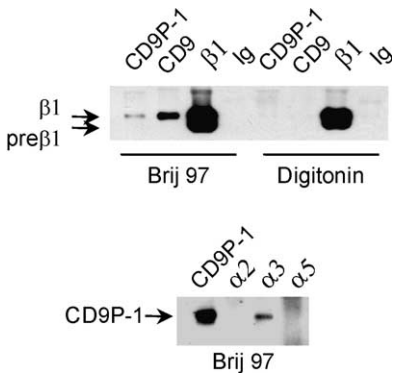

Fig. 3. Presence of CD9P-1 and integrin α3β1 in the same complex. A431 cells were lysed in the presence of Brij97 or digitonin. Immunoprecipitations were performed as indicated on the top of each lane and analyzed by Western blot using biotin-labeled mAbs to β1-integrin (top) or CD9P-1 (bottom).

These data strongly suggest that CD9P-1 and the integrin α3β1 interact only *indirectly* through tetraspanin/tetraspanin interactions.

Buoyant properties of tetraspanins

The tetraspanins were recently found to partition in DRM. To determine whether CD151 could influence the localization of the integrin α3β1 in DRM, Brij97-resistant membranes were isolated by buoyancy at low-density fractions of a bottom-loaded discontinuous sucrose gradient and analyzed by immunoblotting (Fig. 4A). In initial experiments, the density pattern of tetraspanins from HeLa cells was studied and compared to usual controls. As expected, the GPI-anchored molecule CD55, a resident of GPI-microdomains [34], was mainly recovered in low-density fractions (1–3), and CD71, a protein not associated with DRM, was recovered in high-density fractions (5–8). A significant proportion of the tetraspanins CD9, CD81, CD151 (up to ≈30%, Fig. 4A), and CD63 (not shown) were found to partition into low-density fractions, both in lymphoid cells (data not shown) and HeLa cells (Fig. 4), indicating that the tetraspanins can associate with Brij97-resistant membranes. The partition of tetraspanins into low-density fractions did not occur after lysing the cells in Triton X-100 (Fig. 4C), a condition which preserves the GPI-microdomains and disrupts the tetraspanin/tetraspanin complexes. To determine whether the localization of tetraspanins in DRM reflects an association with rafts or with other membrane structures, the cells were treated with Brij97 at 37 °C (instead of 4 °C in standard conditions), to disrupt rafts [34]. The tetraspanins still floated within low-density fractions while raft markers such as CD55 redistributed into high-density fractions (Fig. 4B), indicating that tetraspanins localize in a membrane environment different from rafts: this is further suggested by previous reports failing to put in evidence any association between tetraspanins and raft resident molecules such as GPI-anchored molecules ([32,37] and data not shown) or protein kinases [38]. Also, the tetraspanin-associated HLA-DR molecules (CDw78) showed a restricted repertoire of self-peptides as compared to the raft-associated HLA-DR molecules [39]. These data do not exclude the possibility that under particular circumstances, the tetraspanin complexes and GPI-microdomains could cluster. This might explain a discrepancy between our data and a previous report. Indeed, in murine T cells, a low fraction of CD9 was recently shown to be present in low-density fractions after lysis with Triton X-100, a property shared by other co-stimulatory molecules [40].

CD151 redistributes the integrin α3β1 into DRM

The tetraspanin-associated α3 integrin [13,28], but not the non-associated α5, was also readily detected in the low-density fractions of sucrose gradients (Fig. 4B). In addition, the behavior of α3 paralleled that of the tetraspanins, i.e., α3 was buoyant after lysis with Brij97 at 4 or 37 °C (Fig. 4B) but was fully recovered in the high-density fractions after cholesterol depletion with

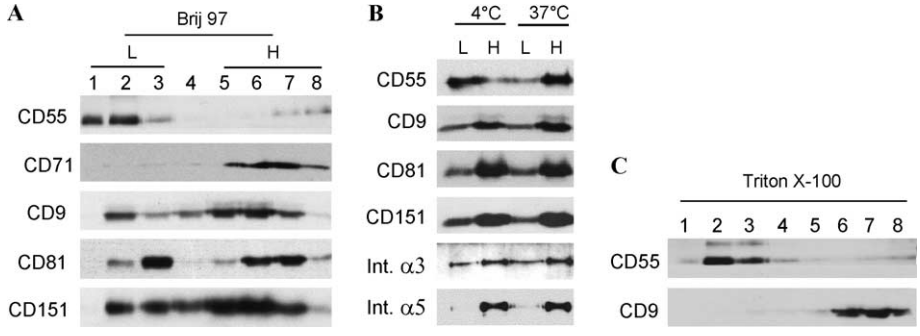

Fig. 4. Association of tetraspanins and α3β1 integrin with detergent-resistant membranes. HeLa cells were lysed and the extracts were directly subjected to equilibrium density gradient centrifugation. Gradient fractions were collected and analyzed by Western blot. The fractions are indicated on the top of each lane. Fractions 1–3 correspond to low-density fractions (L), and 5–8 to high-density fractions (H). (A) Lysis in Brij97 lysis buffer at 4 °C. (B) Lysis in Brij97 lysis buffer either at 4 or at 37 °C. (C) Lysis in Triton X-100 lysis buffer.

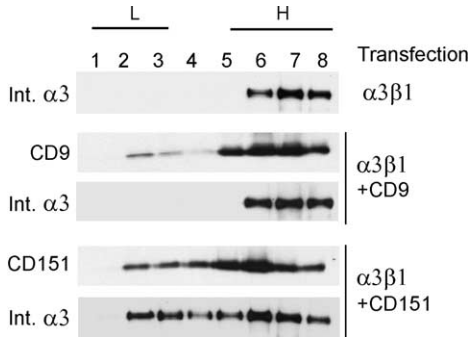

Fig. 5. CD151 allows the redistribution of the α3β1 integrin into DRM. Daudi cells expressing the integrin α3β1 with or without CD151 or CD9 were lysed at 4 °C in the presence of Brij97 and the extracts were directly subjected to equilibrium density gradient centrifugation. Gradient fractions were collected and analyzed by Western blot. The fractions are indicated on the top of each lane. Fractions 1–3 correspond to low-density fractions and 5–8 to high-density fractions.

MβCD (data not shown). The buoyancy of α3β1 integrin is dependent on its association with tetraspanins. Indeed, in transfected Daudi cells, this integrin distributed into the low-density fractions only when CD151 was expressed and the integrin associated with other tetraspanins (Fig. 5).

In previous studies, other tetraspanin partners were shown to partition into low-density fractions of discontinuous sucrose gradients [27,30] and it is likely that their partition also depends on the association with tetraspanins.

A model for the tetraspanin web

The data presented in this paper strengthen a model of a tetraspanin web organized in several different levels. At the first level, each tetraspanin forms primary complexes with a limited number of molecular partners which are best revealed after digitonin extraction [28,29]. These primary complexes most likely involve direct protein/protein interaction as demonstrated for the CD151/integrin and CD9/CD9P-1 pairs [29,31,32]. They are probably independent from lipid interactions, since they can be observed after extraction with digitonin [24,29,31,41], which does not extract any cholesterol and very few lipids [42]. At the second level, several primary complexes would assemble through tetraspanin/tetraspanin interaction, in a cholesterol-containing environment (submitted for publication), to form higher-ordered complexes. It is possible that this assembly is facilitated by the 4 hydrophobic (presumably transmembrane) domains of the tetraspanins as well as their palmitoylation [43–45]. As suggested by Claas et al. [37], the observation that a certain number of molecules can be observed only after lysis in detergents that are milder than Brij97 suggests the possible existence of a third level of interaction. Because tetraspanin to tetraspanin interactions are readily observed in Brij97, it is likely that the basis for this third level of interaction is different from the tetraspanin/partner pair model. The data presented in this paper further suggest that tetraspanins function as ''organizers'' of multimolecular complexes [12] that may represent regulatory units controlling particular properties of tetraspanin's partner molecules. Diseases associated with a loss or a decreased expression of tetraspanins may relate to the disorganization of these complexes.

Acknowledgments

We are grateful to Dr. F. Altruda, Dr. J. Bréard, Dr. M. Hemler, Dr. F. Lanza, Dr. P. Natali, and Dr. Zardi for the generous gift of cDNA and antibodies. The KIAA1436 cDNA was provided by the Kazusa DNA research institute, Japan. This work was supported by the Association Nouvelles Recherches Biomédicales, by the Association pour la Recherche contre le Cancer, and by the Institut de Cancérologie et d'Immunogénétique. S.C. is supported by a fellowship from the Association pour la Recherche contre le Cancer.

References

[1] C. Boucheix, E. Rubinstein, Tetraspanins, Cell. Mol. Life Sci. 58 (2001) 1189–1205.
[2] F. Le Naour, E. Rubinstein, C. Jasmin, M. Prenant, C. Boucheix, Severely reduced female fertility in CD9-deficient mice, Science 287 (2000) 319–321.
[3] K. Miyado, G. Yamada, S. Yamada, H. Hasuwa, Y. Nakamura, F. Ryu, K. Suzuki, K. Kosai, K. Inoue, A. Ogura, M. Okabe, E. Mekada, Requirement of CD9 on the egg plasma membrane for fertilization, Science 287 (2000) 321–324.
[4] K. Kaji, S. Oda, T. Shikano, T. Ohnuki, Y. Uematsu, J. Sakagami, N. Tada, S. Miyazaki, A. Kudo, The gamete fusion process is defective in eggs of Cd9-deficient mice, Nat. Genet. 24 (2000) 279–282.
[5] R. Zemni, T. Bienvenu, M.C. Vinet, A. Sefiani, A. Carrie, P. Billuart, N. McDonell, P. Couvert, F. Francis, P. Chafey, F. Fauchereau, G. Friocourt, V. Portes, A. Cardona, S. Frints, A. Meindl, O. Brandau, N. Ronce, C. Moraine, H. Bokhoven, H.H. Ropers, R. Sudbrak, A. Kahn, J.P. Fryns, C. Beldjord, J. Chelly, A new gene involved in X-linked mental retardation identified by analysis of an X;2 balanced translocation, Nat. Genet. 24 (2000) 167–170.
[6] P. Pileri, Y. Uematsu, S. Campagnoli, G. Galli, F. Falugi, R. Petracca, A.J. Weiner, M. Houghton, D. Rosa, G. Grandi, S. Abrignani, Binding of hepatitis C virus to CD81, Science 282 (1998) 938–941.
[7] O. Silvie, E. Rubinstein, J.F. Franetich, M. Prenant, E. Belnoue, L. Renia, L. Hannoun, W. Eling, S. Levy, C. Boucheix, D. Mazier, Hepatocyte CD81 is required for *Plasmodium falciparum* and *Plasmodium yoelii* sporozoite infectivity, Nat. Med. 9 (2003) 93–96.
[8] S. Ikeyama, M. Koyama, M. Yamaoko, R. Sasada, M. Miyake, Suppression of cell motility and metastasis by transfection with human motility-related protein (MRP-1/CD9) DNA, J. Exp. Med. 177 (1993) 1231–1237.
[9] J.T. Dong, P.W. Lamb, C.W. Rinker-Schaeffer, J. Vukanovic, T. Ichikawa, J.T. Isaacs, J.C. Barrett, KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2, Science 268 (1995) 884–886.
[10] C. Boucheix, H.T. Duc, C. Jasmin, E. Rubinstein, Tetraspanins and malignancy, Exp. Rev. Mol. Med., http://www-ermm.cbcu.cam.ac.uk/01002381h.htm, 2001.

[11] P. Angelisova, I. Hilgert, V. Horejsi, Association of four antigens of the tetraspans family (CD37, CD53, TAPA-1, and R2/C33) with MHC class II glycoproteins, Immunogenetics 39 (1994) 249–256.
[12] E. Rubinstein, F. Le Naour, C. Lagaudrière, M. Billard, H. Conjeaud, C. Boucheix, CD9, CD63, CD81, and CD82 are components of a surface tetraspan network connected to HLA-DR and VLA integrins, Eur. J. Immunol. 26 (1996) 2657–2665.
[13] F. Berditchevski, M.M. Zutter, M.E. Hemler, Characterization of novel complexes on the cell surface between integrins and proteins with 4 transmembrane domains (TM4 proteins), Mol. Biol. Cell 7 (1996) 193–207.
[14] H.T. Maecker, S.C. Todd, S. Levy, The tetraspanin superfamily: molecular facilitators, FASEB J. 11 (1997) 428–442.
[15] F. Berditchevski, K.F. Tolias, K. Wong, C.L. Carpenter, M.E. Hemler, A novel link between integrins, transmembrane-4 superfamily proteins (CD63 and CD81), and phosphatidylinositol 4-kinase, J. Biol. Chem. 272 (1997) 2595–2598.
[16] X.A. Zhang, A.L. Bontrager, M.E. Hemler, Transmembrane-4 superfamily proteins associate with activated protein kinase C (PKC) and link PKC to specific β(1) integrins, J. Biol. Chem. 276 (2001) 25005–25013.
[17] E. Rubinstein, F. Le Naour, M. Billard, M. Prenant, C. Boucheix, CD9 antigen is an accessory subunit of the VLA integrin complexes, Eur. J. Immunol. 24 (1994) 3005–3013.
[18] A.R.E. Shaw, A. Domanska, A. Mak, A. Gilchrist, K. Dobler, L. Visser, S. Poppema, L. Fliegel, M. Letarte, B.J. Willett, Ectopic expression of human and feline CD9 in a human B cell line confers b1 integrin-dependent motility on fibronectin and laminin substrates and enhanced tyrosine phosphorylation, J. Biol. Chem. 270 (1995) 24092–24099.
[19] C. Lagaudrière-Gesbert, F. Le Naour, S. Lebel-Binay, M. Billard, E. Lemichez, C. Boucheix, H. Conjeaud, E. Rubinstein, Functional analysis of four tetraspans, CD9, CD53, CD81, and CD82, suggests a common role in costimulation, cell adhesion, and migration: only CD9 upregulates HB-EGF activity, Cell. Immunol. 182 (1997) 105–112.
[20] M. Yanez-Mo, A. Alfranca, C. Cabanas, M. Mazaruela, R. Tejedor, M.A. Ursa, L.K. Ashman, M.O. de Landazuri, F. Sanchez-Madrid, Regulation of endothelial cell motility by complexes of tetraspan molecules CD81/TAPA-1 and CD151/PETA-3 with α3β1 integrin localized at endothelial lateral junctions, J. Cell Biol. 141 (1998) 791–804.
[21] P.M. Sincock, S. Fitter, R.G. Parton, M.C. Berndt, J.R. Gamble, L.K. Ashman, PETA-3/CD151, a member of the transmembrane 4 superfamily, is localised to the plasma membrane and endocytic system of endothelial cells, associates with multiple integrins and modulates cell function, J. Cell Sci. 112 (1999) 833–844.
[22] T. Sugiura, F. Berditchevski, Function of α3β1-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 (1999) 1375–1389.
[23] M.R. Schick, V.Q. Nguyen, S. Levy, Anti-TAPA-1 antibodies induce protein tyrosine phosphorylation that is prevented by increasing intracellular thiol levels, J. Immunol. 151 (1993) 1918–1925.
[24] G. Horváth, V. Serru, D. Clay, M. Billard, C. Boucheix, E. Rubinstein, CD19 is linked to the integrin-associated tetraspans CD9, CD81, and CD82, J. Biol. Chem. 273 (1998) 30537–30543.
[25] R.L. Yauch, F. Berditchevski, M.B. Harler, J. Reichner, M.E. Hemler, Highly stoichiometric, stable, and specific association of integrin α3β1 with CD151 provides a major link to phosphatidylinositol 4-kinase, and may regulate cell migration, Mol. Biol. Cell 9 (1998) 2751–2765.
[26] K.L. Clark, Z. Zeng, A.L. Langford, S.M. Bowen, S.C. Todd, Pgrl is a major cd81-associated protein on lymphocytes and distinguishes a new family of cell surface proteins, J. Immunol. 167 (2001) 5115–5121.
[27] C.S. Stipp, T.V. Kolesnikova, M.E. Hemler, EWI-2 Is a Major CD9 and CD81 partner and member of a novel Ig protein subfamily, J. Biol. Chem. 276 (2001) 40545–40554.
[28] V. Serru, F. Le Naour, M. Billard, D.O. Azorsa, F. Lanza, C. Boucheix, E. Rubinstein, Selective tetraspan/integrin complexes (CD81/α4β1, CD151/α3β1, CD151/α6β1) under conditions disrupting tetraspan interactions, Biochem. J. 340 (1999) 103–111.
[29] S. Charrin, F. Le Naour, M. Oualid, M. Billard, G. Faure, S.M. Hanash, C. Boucheix, E. Rubinstein, The major CD9 and CD81 molecular partner: identification and characterization of the complexes, J. Biol. Chem. 276 (2001) 14329–14337.
[30] C.S. Stipp, D.J. Orlicky, M.E. Hemler, FPRP: a major, highly stoichiometric, highly specific CD81 and CD9-associated protein, J. Biol. Chem. 276 (2001) 4853–4862.
[31] R.L. Yauch, A.R. Kazarov, B. Desai, R.T. Lee, M.E. Hemler, Direct extracellular contact between integrin α(3)β(1) and TM4SF protein CD151, J. Biol. Chem. 275 (2000) 9230–9238.
[32] S. Lozahic, D. Christiansen, S. Manie, D. Gerlier, M. Billard, C. Boucheix, E. Rubinstein, CD46 (membrane cofactor protein) associates with multiple β1 integrins and tetraspans, Eur. J. Immunol. 30 (2000) 900–907.
[33] K. Simons, D. Toomre, Lipid rafts and signal transduction, Nat. Rev. Mol. Cell Biol. 1 (2000) 31–39.
[34] V. Horejsi, K. Drbal, M. Cebecauer, J. Cerny, T. Brdicka, P. Angelisova, H. Stockinger, GPI-microdomains: a role in signalling via immunoreceptors, Immunol. Today 20 (1999) 356–361.
[35] D.O. Azorsa, S. Moog, J.P. Cazenave, F. Lanza, A general approach to the generation of monoclonal antibodies against members of the tetraspanin superfamily using recombinant GST fusion proteins, J. Immunol. Methods 229 (1999) 35–48.
[36] A. Bartolazzi, R. Fraioli, G. Tarone, P.G. Natali, Generation and characterization of the murine monoclonal antibody M-KID 2 to VLA-3 integrin, Hybridoma 10 (1991) 707–720.
[37] C. Claas, C.S. Stipp, M.E. Hemler, Evaluation of prototype TM4SF protein complexes and their relation to lipid rafts, J. Biol. Chem. 276 (2001) 4853–4862.
[38] J. Cerny, H. Stockinger, V. Horejsi, Noncovalent associations of T lymphocyte surface proteins, Eur. J. Immunol. 26 (1996) 2335–2343.
[39] H. Kropshofer, S. Spindeldreher, T.A. Rohn, N. Platania, C. Grygar, N. Daniel, A. Wolpl, H. Langen, V. Horejsi, A.B. Vogt, Tetraspan microdomains distinct from lipid rafts enrich select peptide-MHC class II complexes, Nat. Immunol. 3 (2002) 61–68.
[40] Y. Yashiro-Ohtani, X.Y. Zhou, K. Toyo-Oka, X.G. Tai, C.S. Park, T. Hamaoka, R. Abe, K. Miyake, H. Fujiwara, Non-CD28 costimulatory molecules present in T cell rafts induce T cell costimulation by enhancing the association of TCR with rafts, J. Immunol. 164 (2000) 1251–1259.
[41] T.F. Tedder, M. Inaoki, S. Sato, The CD19–CD21 complex regulates signal transduction thresholds governing humoral immunity and autoimmunity, Immunity 6 (1997) 107–118.
[42] P. Banerjee, J.B. Joo, J.T. Buse, G. Dawson, Differential solubilization of lipids along with membrane proteins by different classes of detergents, Chem. Phys. Lipids 77 (1995) 65–78.
[43] S. Charrin, S. Manie, M. Oualid, M. Billard, C. Boucheix, E. Rubinstein, Differential stability of tetraspanin/tetraspanin interactions: role of palmitoylation, FEBS Lett. 516 (2002) 139–144.
[44] X. Yang, C. Claas, S.K. Kraeft, L.B. Chen, Z. Wang, J.A. Kreidberg, M.E. Hemler, Palmitoylation of tetraspanin proteins: modulation of CD151 lateral interactions, subcellular distribution, and integrin-dependent cell morphology, Mol. Biol. Cell 13 (2002) 767–781.
[45] F. Berditchevski, E. Odintsova, S. Sawada, E. Gilbert, Expression of the palmitoylation-deficient CD151 weakens the association of α3β1 integrin with the tetraspanin-enriched microdomains and affects integrin-dependent signaling, J. Biol. Chem. 277 (2002) 36991–37000.