The Roles of Tetraspanins in HIV-1 Replication

Markus Thali

Contents

Abstract Tetraspanins are small integral membrane proteins that are known to control a variety of cellular processes, including signaling, migration and cell–cell fusion. Research over the past few years established that they are also regulators of various steps in the HIV-1 replication cycle, but the mechanisms through which these proteins either enhance or repress virus spread remain largely unknown.

M. Thali
Department of Microbiology and Molecular Genetics, College of Medicine and CALS, University of Vermont, 318 Stafford Hall, 95 Carrigan Drive, Burlington, VT 05405-0084
e-mail: markus.thali@uvm.edu

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1 Introduction

Virus proteomics, i.e., the mapping of interactions that take place between cellular and viral proteins on a global, whole cell scale, or based on analyses of cellular components incorporated into virions, has led to the identification of cellular proteins, and indeed entire cellular pathways, that are critical for the propagation of HIV-1 and other retroviruses (for recent overviews, e.g., Goff 2007, 2008). The most straightforward approach leading to the identification of such proteins/pathways is the analysis of virion content. Fifteen years ago such an analysis hinted at the possibility that tetraspanins play a role in the replication of this virus by revealing that a member of the tetraspanin family is specifically incorporated into HIV-1 particles (Orentas and Hildreth 1993). Over the past 5 years, various investigators have followed up on this early study, primarily by further characterizing the presence of tetraspanins at the viral budding site. However, of the two dozen reports that now link tetraspanins with HIV-1 replication steps (see Table 1), so far only a few document functional roles in virus propagation. Therefore, and as I will discuss in this review, while we now know that tetraspanins have regulatory roles during HIV-1 replication, we are only at the very beginning of understanding exactly how these proteins function during transmission, and thus ultimately propagation and pathogenesis, of HIV-1.

In the following, I will first briefly summarize what we know about cellular tetraspanin functions. Subsequently, I will review the existing literature on the interaction between tetraspanins and HIV-1. Finally, I will discuss potential mechanisms through which tetraspanins exert their functions and, though only very briefly, I will also touch upon the role of tetraspanins in the replication cycles of other viruses.

Table 1 Tetraspanins at different stages of the HIV-1 replication cycle

Assembly	Ruiz-Mateos, Pelchen-Matthews et al. 2008
Orentas and Hildreth 1993	Grigorov, Attuil-Audenis et al. 2009
Gluschankof, Mondor et al. 1997	
Meerloo, Sheikh et al. 1993	**Transmission to target cells & virus-cell and cell-cell fusion**
Raposo, Moore et al. 2002	Gordon-Alonso, Yanez-Mo et al. 2006
Nydegger, Foti et al. 2003	Sato, Aoki et al. 2007
Pelchen-Matthews, Kramer et al. 2003	Singethan, Muller et al. 2008
Nydegger, Khurana et al. 2006	Weng, Krementsov et al. 2009
Booth, Fang et al. 2006	Krementsov, Weng et al. 2009
Grigorov, Arcanger et al. 2006	
Welsch, Keppler et al. 2007	**Susceptibility of potential target cells**
Deneka, Pelchen-Matthews et al. 2007	von Lindern, Rojo et al. 2003
Jolly and Sattentau 2007	Ho, Martin et al. 2006
Garcia, Nikolic et al. 2008	Yoshida, Kawano et al. 2008
Turville, Aravantinou et al. 2008	
	Viral gene expression in newly infected cells
Release	Tardif and Tremblay 2005
Khurana, Krementsov et al. 2007	
Chen, Dziuba et al. 2008	

2 Tetraspanins: Organizers of Membrane-Based Processes

Tetraspanins form a diverse family of small (20–30 kDa, not including mass contributed by glycosylation) membrane proteins that compromises 33 members in mammals (Hemler 2005). Consistent with their involvement in controlling membrane-based processes such as signaling, adhesion and cell–cell fusion, tetraspanins have emerged at the transition from unicellular to multicellular organisms and have since undergone intense evolution (Huang et al. 2005).

2.1 Structure and Subcellular Distribution of Tetraspanins

Tetraspanins contain short cytoplasmic N- and C-termini and one short inner loop that separates a small (SEL) from a large (LEL) extracellular loop. A recent cryoelectron microscopic analysis together with molecular modeling studies revealed that tetraspanins, due to close juxtapositioning of the four transmembrane segments, span the lipid bilayer as compact, rod-shaped structures. Plasma membrane-based tetraspanins are relatively "invisible" from the outside, as they protrude only approximately 5 nms. This probably explains why only very few of them do serve as receptors. However, as described elegantly by others, *"with the lower half of the bundle embedded in the lipid bilayer, the tetraspanins may serve as pilings in the lipid sea, ideal for docking other transmembrane proteins"* (Min et al. 2006). Indeed, the consensus reached by researchers analyzing these proteins is that tetraspanins function primarily as scaffold proteins that laterally organize various membrane-based cellular functions (e.g., Stipp et al. 2003; Hemler 2005; Levy and Shoham 2005a, b). Biochemical analyses over the past 15 years have demonstrated that they form homodimers and that they also tightly associate with other transmembrane proteins, including specific integrins and members of the immunoglobulin superfamily. These associations link molecular events taking place within membranes with membrane-peripheral signaling complexes and the cytoskeleton. Recent high resolution ultrastructural (electron microscopy) and/or fluorescence microscopy studies performed in our lab as well as by others (Nydegger et al. 2006; Unternaehrer et al. 2007), for the first time visualized these up to few hundred nanometer-wide, tetraspanin-mediated assemblages of proteins (TEMs: tetraspanin-enriched microdomains). Importantly, while these initial studies were performed in fixed cells, using bivalent antibody-based detection (which can exaggerate the discreteness of microdomains due to antibody-induced microclustering), two very recent analyses of tetraspanin distribution at the single molecule level in live cells clearly confirmed that these proteins are locally concentrated, thus forming submicron-sized "interaction platforms" (Barreiro et al. 2008; Espenel et al. 2008). One of these latter studies (Espenel et al. 2008) also confirmed what was previously reported by others (e.g., Yang et al. 2004), i.e., that TEMs are clearly distinct from lipid rafts (now also called membrane rafts).

2.2 Cellular Functions of Tetraspanins

Based on analyses of amino acid sequences of the LEL, the segment known to be the primary binding site for tetraspanin-associated proteins, the 33 members of this family can be subdivided into four subgroups (Seigneuret et al. 2001). Members of the same subgroup apparently can partially fulfill each other's role, in case a specific tetraspanin is ablated (e.g., in mouse knockout systems), suggesting a certain degree of redundancy. While such redundancy provides obvious benefits to organisms, it complicates genetic analyses of functions for individual members of a protein family, and thus not surprisingly, only in a few cases has the deletion of a tetraspanin gene resulted in dramatic phenotypes, such as the loss of fertility in CD9 knock-out mice, or retinal degeneration in peripherin (tetraspanin 22) knock-out mice (reviewed, e.g., in Hemler 2005; see also Fradkin et al. 2002). Nevertheless, genetic studies clearly revealed that tetraspanins play regulatory roles in numerous membrane-based processes and several recent reviews provide an overview of the various functions (Boucheix et al. 2001; Wright et al. 2004; Levy and Shoham 2005a, b; Hemler 2008). Here, I will merely summarize what we know so far about the involvement of two members of subgroup 1 (Seigneuret et al. 2001) of the tetraspanin family, CD9 and CD81, in the regulation of membrane fusion, because, as will be discussed later, these two members of the tetraspanin family, and also CD63 (subgroup 2b), are co-regulators of HIV-1-induced virus–cell or cell–cell fusion.

Importantly, like other scaffold proteins, tetraspanins can both enhance or repress the activities of other cellular proteins. For example, the expression of specific cell surface tetraspanins has been shown to either enhance or slow down cell migration, depending on the conditions. Similarly, signaling cascades can either be augmented or dampened by these proteins, and, as will be described in the following, the same tetraspanins can also act as either positive or negative regulators of cellular fusion processes. CD9 and CD81 were documented to promote myotube formation through their enhancement of muscle cell fusion (Tachibana and Hemler 1999). As was already apparent at that time, these two members of the tetraspanin family do not achieve this through binding to partner proteins on adjacent cells and they do not themselves function as fusion proteins. Rather, they regulate myotube formation through the organization *in cis* of associated, so far still unidentified, cellular fusogens. Interestingly, 4 years after having been recognized to be fusion promoters, the same two tetraspanins (CD9 and CD81) were found to negatively regulate the fusion of another type of somatic cell: Mekada and colleagues showed that the formation of multinuclear phagocytes which ingest infectious microbes, cell fragments etc, is enhanced in CD9- and CD81-null mice (Takeda et al. 2003). This was surprising because, by then, these tetraspanins had also been implicated, besides enhancing myotube formation, in promoting the fusion of germ line cells: CD9 knockout mice oocytes are unable to fuse with sperm (Le Naour et al. 2000; Miyado et al. 2000), and overexpression of CD81 in CD9 knockout mice can partially compensate for CD9's fusion promoting

function (Kaji et al. 2002). Importantly, comparable to the situation in muscle cells, the expression of CD9 in oocytes is required not because tetraspanin acts as cellular fusogen, but because this protein laterally organizes (a) cellular fusion protein(s) (Ziyyat et al. 2006) that interacts in trans with sperm-based proteins, such as Izumo (Inoue et al. 2005), possibly through interactions mediated by its LEL (e.g., Zhu et al. 2002; Higginbottom et al. 2003). Finally, and most intriguingly (at least for virologists), a very recent report demonstrates that CD9 fulfills its fusion control function not through its presence at the oocyte surface but rather upon incorporation into (exosome-like) vesicles that are shed from the oocytes (Miyado et al. 2008).

3 Tetraspanins are Regulators of HIV-1 Replication

3.1 Tetraspanins are present at viral exit sites

The analysis of virus lipid content (Aloia et al. 1993) guided subsequent studies revealing that HIV-1 buds through membrane domains enriched in distinct lipids (reviewed, e.g., in Ono and Freed 2005; see also Brugger et al. 2006). Similarly, three early analyses of cellular proteins incorporated into HIV-1 which revealed the incorporation of a tetraspanin, CD63, into viral particles (Meerloo et al. 1992, 1993; Orentas and Hildreth 1993), foreshadowed what is now well established: HIV-1 exits at segments of cellular membrane that are enriched in tetraspanins. Importantly, one of these early studies, using a solid phase virus capture assay to identify cellular proteins incorporated into HIV-1 particles, combined with flow cytometric analysis of the host cell membrane, already documented that CD63 incorporation into virions is a non-random process. As also shown for the major histocompatibility antigen HLA-DR, this tetraspanin is specifically incorporated into HIV-1 particles released from T lymphocytes. However, except for another study of host cell protein incorporation into HIV-1 virions, which confirmed that CD63 is enriched in infectious particles (Gluschankof et al. 1997), to the best of my knowledge, nobody followed up on these early findings until about 5 years ago.

Tetraspanins were "re-identified" as potential players in HIV-1 replication when different investigators started scrutinizing (primarily using fluorescence and electron microscopy) where exactly HIV-1 buds from cells and how this virus recruits the cellular ESCRT machinery that mediates its release from cells (for reviews, e.g., Freed 2004; Morita and Sundquist 2004; Bieniasz 2006). Initially, it was shown that HIV-1 (and also SIV) components, particularly the viral envelope glycoprotein, Env, at least under certain physiological conditions and in certain cell types, can traffic through sections of the cellular endocytic system (Hunter and Swanstrom 1990; Rowell et al. 1995; Sauter et al. 1996; Ohno et al. 1997; Boge et al. 1998; reviewed in Marsh and Pelchen-Matthews 2000), where the tetraspanin CD63 was known to primarily reside. Further support for the idea that HIV-1 exit sites may share certain characteristics, may perhaps even be somehow related to endosomal

membranes, came from the finding that TSG101, a component of the ESCRT I complex which is required for the formation of intralumenal vesicles of late endosomes/multivesicular bodies (LEs/MVBs), is critical for HIV–1 release. Further, an electron microscopy study by Raposo and colleagues suggested that in macrophages HIV-1 buds into LEs/MVBs (Raposo et al. 2002). Promptly, two studies published in 2003 documented that this virus acquires CD63 (and also CD81 and CD82 if produced in macrophages), when it buds through either what appeared at that time to be LE/MVB membranes of macrophages (Pelchen-Matthews et al. 2003) or when it buds through the plasma membrane in HeLa cells (Nydegger et al. 2003). The latter finding was puzzling because in HeLa cells CD63 has extremely low abundance at the plasma membrane. Nevertheless, based on those data, we hypothesized that this tetraspanin, perhaps together with other members of this family, accumulates at relatively discrete plasma membrane microdomains, and in a subsequent study we indeed provided a first visualization of TEMs, as mentioned above (Nydegger et al. 2006). This analysis, together with biochemical, fluorescence microscopy and again electron microscopy analyses by several other groups, unequivocally confirmed and extended the earlier studies by showing that HIV-1 exits through membrane microdomains enriched in the tetraspanins CD9, CD63, CD81 and CD82 in epithelial cells, T lymphocytes, macrophages and dendritic cells (Booth et al. 2006; Grigorov et al. 2006; Nydegger et al. 2006; Deneka et al. 2007; Jolly and Sattentau 2007; Welsch et al. 2007; Garcia et al. 2008; Turville et al. 2008). Last, but certainly not least, a virion proteomics study of cellular proteins incorporated into HIV-1 released from macrophages not only again revealed the presence of these four tetraspanins, it also reported the incorporation of two additional members of the family (CD53 and tetraspanin 14) (Chertova et al. 2006). How TEMs form and exactly when and how HIV-1 components start interacting with tetraspanins remains to be elucidated. We originally speculated that CD63-containing TEMs at the plasma membrane derive from TEMs that originate in LEs/MVBs and that the viral components perhaps even associate with these domains while they are still part of these organelles (Nydegger et al. 2003). Such a scenario would appear plausible, as it has been documented that the limiting membrane of LE/MVB, upon movement of these organelles to the cell surface, can be inserted as patches into the plasma membrane (Jaiswal et al. 2004). This idea received support from the findings that Rab9 and AP3, cellular proteins implicated in trafficking to and from LEs/MVBs, are necessary for efficient HIV-1 release (Dong et al. 2005; Murray et al. 2005). However, the fact that a very considerable fraction of CD63 traffics to the cell surface before reaching its final destination (LEs/MVBs) (Janvier and Bonifacino 2005), together with other evidence, also makes it likely that the HIV-1 components start associating with tetraspanins only at the plasma membrane, and not while these membrane proteins are carried along vesicles. Indeed, data presented in a recent high resolution (TIRF) microscopy analysis (Jouvenet et al. 2008) also support this idea. The investigators of that study reported that, surprisingly, they did not detect any CD63 association with budding virions at the plasma membrane, while they observed large amounts of this tetraspanin in intracellular vesicles (sometimes containing Gag) moving near

the sites of viral morphogenesis and release, but never fusing with the plasma membrane. However, as previously documented (Nydegger et al. 2006), surface CD63 cannot be detected in settings when total cellular CD63 is visualized (either by GFP-tagging or by overall staining). Thus, the failure by Jouvenet et al. to detect CD63 in viral budding structures (Jouvenet et al. 2008) can presumably be explained technically: the strong fluorescence signal for CD63 that emanated from membrane-proximal vesicles densely packed with this tetraspanin probably prevented the detection of much fainter signals that emanate from the relatively low amounts of CD63 associated with HIV-1 budding structures.

Given their presence at HIV-1 exit sites, are tetraspanins gatekeepers, do they facilitate or even promote particle release? Two recently published studies (Sato et al. 2007; Ruiz-Mateos et al. 2008) in which one tetraspanin (CD63) was either ablated or overexpressed, negatively answers this question for HIV-1 release from macrophages and 293 T cells, and our own investigations of release from HeLa cells and T lymphocytes lead to the same conclusion (Krementsov et al., Retrovirology, in press), though one recent study reports that decreased CD63 expression in macrophages results in reduced HIV-1 particle output (Chen et al. 2008), and an even more recent study correlates reduced levels of CD81 with decreased virus release from Molt T cells (Grigorov et al. 2009). Again arguing against the idea that tetraspanins act as general release factors, recent data from our laboratory demonstrate that CD9 expression can be abrogated without consequences for the rate with which HIV-1 is released from these cells (Krementsov et al., Retrovirology, in press). Initially, this came as a surprise to us, because an incubation of cells producing another lentivirus (feline immunodeficiency virus, FIV, see below) with an anti-feline CD9 antibody (de Parseval et al. 1997), as well as the incubation of HIV-1-producing HeLa cells with an anti-human CD9 antibody (K41) can significantly reduce the rate with which HIV-1 is released from these cells (Khurana et al. 2007). However, as we documented in that latter study, the treatment with K41 resulted in the aggregation of CD9 and other members of the tetraspanin family at cell–cell junctions, thus possibly simply sterically blocking virus release. Altogether, currently available evidence suggests that tetraspanins do not generally act as budding co-factors for HIV-1, though further studies will need to address the question if some of them play a supportive role in certain cell types.

3.2 Tetraspanins in HIV-1 Virions Inhibit Env-Induced Membrane Fusion

If tetraspanins do not act as budding co-factors, why did HIV-1 evolve to exit at membrane segments enriched in these proteins? Given what we now know about the crucial role that CD9 plays in the sperm–egg fusion process, it would have appeared reasonable to hypothesize that tetraspanins, upon incorporation into viral particles, enhance their fusogenicity, e.g., by laterally organizing viral Env.

Indeed, to virologists the recent finding that CD9-bearing exosomes mediate fusion of adjacent cells (sperm and egg, see above) is reminiscent of the phenomenon called "fusion from without" (Bratt and Gallaher 1969); documented for HIV-1 in (Clavel and Charneau 1994): virions that are added to cells in large numbers will act as fusion-bridges, thus promoting the formation of syncytia. However, data recently published by the Koyanagi laboratory (Sato et al. 2007) together with our unpublished observations demonstrate that tetraspanins, if acquired by HIV-1 particles, reduce the fusogenicity of the virions. Indeed, the incorporation of tetraspanins CD9, CD63, CD81, CD82, and CD231 considerably diminishes the infectivity of HIV-1 particles, and these tetraspanins thus act as negative regulators of Env-induced membrane fusion, comparable to how CD9 and CD81 negatively regulate the fusion of monocyte-macrophages. Based on these data, it has been speculated (Sato et al. 2007) that such a fusion-suppressing activity of, e.g., CD63 explains why this tetraspanin is specifically downregulated upon reactivation of chronically infected T lymphocytes, once they increase their virus output (Sato et al. 2007). However, the finding that tetraspanins, despite an overall downregulation from the surface of infected cells (Krementsov et al. Retrovirology, in press), still accumulate at virus release sites (e.g., Jolly and Sattentau 2007) and are still incorporated into virions, as described above, suggests that tetraspanins do not merely act as restriction factors for HIV-1. Rather, combined with the observation that some anti-tetraspanin antibodies appear to negatively affect the alignment of HIV-1 producer and target cells (Jolly and Sattentau 2007), this suggests that they can act as both promoters and inhibitors of HIV-1 transmission. Indeed, as I will lay out below, the role played by tetraspanins expressed at the surface of uninfected or of newly infected cells further supports the idea that these proteins have pleiotropic effects on HIV-1 replication and that these effects can be positive or negative.

3.3 Tetraspanins Regulate HIV-1 Entry and the Transcription of the Viral Genome in Newly Infected Cells

While most of the papers on HIV-1 replication and tetraspanins suggest or describe roles of these proteins during the assembly/release phase of the viral replication cycle, recent reports clearly document that tetraspanins also affect virus replication at the entry phase and upon integration of the viral genome into host chromosomes. Data presented in two studies showed that the treatment of macrophages with either an anti-CD63 antibody (von Lindern et al. 2003) or with recombinant LELs of the tetraspanins CD9, CD63, CD81, and CD151 (Ho et al. 2006) can inhibit HIV-1 entry, probably by blocking a post-binding step. While this suggests positive roles for these tetraspanins in the infection process, CD63, but none of the other tetraspanins analyzed (CD9, CD81, and CD151) was also recently shown to divert the co-receptor CXCR4 from its trafficking to the cell surface, thus reducing its presence there and consequently reducing the susceptibility of cells to HIV-1

(Yoshida et al. 2008). This later report, which suggested that tetraspanins, specifically CD63, negatively regulate HIV-1 replication, is counterbalanced by another report, which documented a potential role for CD81 as a co-stimulatory molecule that enhances the transcription of the newly integrated HIV-1 genome (Tardif and Tremblay 2005). Finally, the authors of a fourth paper remain ambiguous about whether the observed result of CD9 and CD81 downregulation from the surface of potential target cells overall has positive or negative consequences for HIV-1 replication: Sanchez-Madrid and colleagues (Gordon-Alonso et al. 2006) demonstrated that either siRNA-mediated reduction of tetraspanin levels, or antibody-induced interference with normal tetraspanin function in T cells and in CD4-positive target cells, leads to increased fusion of infected and uninfected cells. As will be discussed below, such increased fusion could restrict virus spread, and the presence of tetraspanins at the surface of potential target cells could thus be beneficial for the virus. However, it is also possible that tetraspanins prevent HIV-1 infection because, as was also shown in that paper (Gordon-Alonso et al. 2006), the elimination of tetraspanins from the surface of potential target cells makes these cells more susceptible for HIV-1 infection.

3.4 Tetraspanins Regulate Cell-to-Cell Transmission of HIV-1

As reviewed elsewhere (e.g., Johnson and Huber 2002; Sattentau 2008), and indeed as already proposed 15 years ago (Phillips 1994), HIV-1 apparently is most efficiently transmitted from cell-to-cell, if it is released at cell-cell junctions, into the cleft of what is now called the virological synapse (VS) (Igakura et al. 2003; Jolly et al. 2004; for a review see Piguet and Sattentau 2004; see also Hope 2007). Transmission via the VS may be particularly important in secondary lymphoid organs, which are the major sites of virus replication and where cells can be densely packed, (e.g., in the order of 10^9 cells/ml in lymph nodes, as compared to 10^5–10^6 cells in blood) (see also Sourisseau et al. 2007, for further citations). Evidence that such synaptic transmission takes place in vivo comes from data that document clusters of patient-derived spleen cells that have been infected by HIV-1 derived from the same progeny virus (Cheynier et al. 1994; Hosmalin et al. 2001).

The VS shares certain characteristics with the so-called immunological synapse (IS), which forms between antigen presenting cells and T cells (e.g., Friedl et al. 2005; see also Fackler et al. 2007). Like the IS, the VS represents a transient but nevertheless well-organized functional entity. Comparable to the IS, (and also to the neural synapse), the producer/effector cell, i.e., the presynaptic cell, does not fuse with the target cell (the postsynaptic cell) upon synapse formation. While this lack of fusion may seem normal in the case of the IS and the neural synapse, it certainly comes as a surprise in the case of the VS: why do producer cells, which express Env at their surface, typically not fuse with target cells which express CD4 and chemokine receptors? Why do they not form a syncytium, a multinucleated cell? Adherence without fusion may be explained at least partially by the fact that

unprocessed HIV-1 Gag represses Env fusion activity through an interaction with the cytoplasmic tail of Env (EnvCT) (Murakami et al. 2004; Wyma et al. 2004; Davis et al. 2006; Jiang and Aiken 2006, 2007; for a recent review, see Murakami 2008). Hence, if expressed as part of the virus, most Env becomes fusogenic only when it leaves the producer cell as part of the budding virion, but it can already bind to CD4 before that. One could thus envision a scenario in which a fraction of Env located at the presynapse and still associated with precursor Gag, and thus not fusogenic, triggers adherence of the producer to the target cell, thus allowing for the formation of the synaptic cleft into which virions (with fusion-active Env) can be shed. However, Env-mediated cell–cell fusion is also known to be regulated by cellular proteins, e.g., integrins, present at the surface of producer and target cells (e.g., Ohta et al. 1994; Fais et al. 1996) and it thus seems most likely that viral and cellular proteins, including tetraspanins, act in concert to promote efficient particle transfer by regulating Env-induced membrane fusion. As already mentioned above, CD9 and CD81 prevent syncytium formation through their presence at the virological postsynapse, but they also act at the other side of the VS: our own data demonstrate that the same tetraspanins (CD9 and CD81, and also CD63) prevent HIV-1 Env-induced cell–cell fusion through their presence at the virological presynapse (Weng et al. 2009). Quite likely, such repression of Env-induced fusion by tetraspanins, unlike their fusion inhibitory function in virions (see above), is beneficial for the virus but not for the host, because syncytia, while being able to still produce HIV-1 particles (indeed lots of them; see, e.g., Sylwester et al. 1997), have limited life span and thus cannot continue spreading the virus.

If fusion prevention by tetraspanins is positive for the virus, how can this be reconciled with the finding that tetraspanin incorporation into budding HIV-1 particles reduces their infectivity and is thus detrimental to virus replication and spread? It seems reasonable to assume that fusion regulation at the VS, as well as other transmission related processes that take place at that site, depends on proper spatio-temporal organization of the synapse. Spatial organization of the synapse is now well documented for the IS (e.g., Kaizuka et al. 2007) which, at least in its more stable form (Friedl et al. 2005), has a central zone known as the cSMAC (central supramolecular activation complex) that contains the T cell receptor (TCR), co-stimulatory molecules and signaling components, and an outer ring of proteins known as the pSMAC (peripheral SMAC), comprised of adhesion molecules such as the ICAM-1-LFA-1 pair. Interestingly, a similar localization of ICAM-1 was reported for a VS-like structure that formed when CD4-positive T cells adhered to coverslips coated with gp120 and ICAM-1, with ICAM-1 forming a ring around gp120 that accumulated in the center (Vasiliver-Shamis et al. 2008). A scenario that would reconcile the opposing effects of fusion repression by tetraspanins (negative for the virus because it reduces its infectivity – positive because it prevents syncytium formation) would see tetraspanins, like the adhesion molecules, accumulating preferentially at the VS periphery, where their presence would prevent Env molecules from initiating the fusion of pre- and postsynaptic cells, while the center of the VS, where HIV-1 may bud preferentially, would be relatively deserted by these proteins, thus allowing for the formation of particles

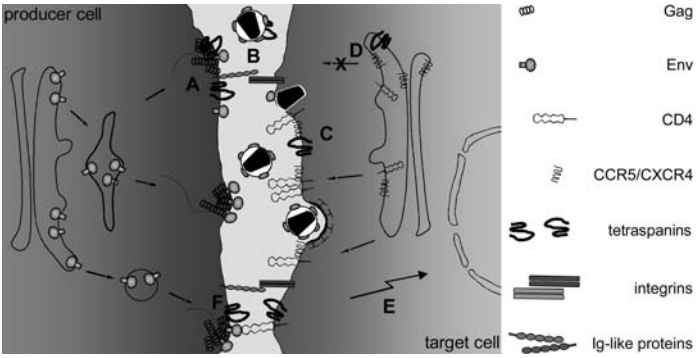

Fig. 1 Tetraspanin functions before, during and after transmission of HIV-1 particles. (A) Formation of viral exit gateways. (B) Incorporation into virions and reduction of infectivity. (C) Repression of Env-mediated virus-cell and cell-cell fusion through interactions with the receptor/coreceptor complex. (D) Reduction of susceptibility to HIV-1 infection through interference with CXCR4 transport to the cell surface. (E) Costimulation of HIV-1 gene expression. (F) Prevention of syncytia formation through interactions with Env in producer cells (see text for details)

with (relatively) few tetraspanins (see Fig. 1 for a scheme of the VS). Examinations of the spatial organization of the VS will allow testing this hypothetical distribution. An alternative, simpler explanation would envision that the virus downregulates tetraspanins to an optimal level that enhances the infectivity of the virions but which still prevents syncytia formation.

3.5 How do Tetraspanins Regulate HIV-1 Entry, Viral Protein Expression, and Env/Receptor-Mediated Fusion Processes?

3.5.1 Tetraspanin Functions in Potential Target Cells and in Newly Infected Cells

While CD63 has been shown to divert CXCR4 from reaching the cell surface, thus preventing infection of cells by HIV-1, it remains to be analyzed if CD63 fulfills this chaperoning function through direct interaction with CXCR4, and if so, where it starts to interact with the coreceptor for HIV-1. Even less is known about the mechanisms with which tetraspanins at the surface of target cells repress fusion of the target cell membrane with the membrane of bound virions and/or bound producer cells. While CD81 is known to associate with CD4 (Imai et al. 1995), downregulation of this HIV-1 receptor apparently is not responsible for the observed fusion repressor function of CD9 and CD81 (Gordon-Alonso et al. 2006). Comparably, receptor/co-receptors in potential target cells are not downregulated upon incubation of these cells with LEL, which inhibits virus entry into macrophages. It seems plausible, however, that tetraspanin knockdowns in T lymphocytes

or incubation of these cells with anti-tetraspanin antibodies, as well as LEL treatment of macrophages, either prevent conformational changes that need to take place for fusion to ensue, or that they alter the microenvironment of the receptors/coreceptors. Such lateral reorganizations may also be at play when CD81 acts as co-stimulator of HIV-1 gene transcription. Since CD81 is known to associate with CD4, one could speculate that an engagement of CD4 by its counter receptor (Env, either on virions or on producer cells) triggers local protein translocations or conformational changes in target cell signaling complexes.

3.5.2 Repression of Fusion Triggered by Virion-Associated or Producer Cell-Associated Env

As described above, we know very little about how tetraspanins regulate infection and post-infection events in lymphocytes. We are even more ignorant about tetraspanin functions in HIV-1 producer cells and in virions. And while these cellular membrane proteins have been established as important players in the replication cycle of other viruses, e.g., in hepatitis C virus (HCV) entry, apparently we cannot extrapolate to HIV-1 from that knowledge. CD81 serves as coreceptor for HCV (e.g., Kapadia et al. 2007; Brazzoli et al. 2008), but the interaction of HCV's envelope glycoprotein E2 with CD81 so far is one of only two cases where a tetraspanin directly interacts with a protein situated in trans, i.e., on the plasma membrane of an adjacent cell (or on the viral membrane). Even the role that the tetraspanin CD82 plays in the replication cycle of another retrovirus (HTLV-1) may be distinct from how tetraspanins regulate HIV-1-induced fusion processes (both virus- or cell-associated fusion processes): while a (probably direct) interaction between HTLV-1 Gag and CD82 was reported to take place (Mazurov et al. 2007), HTLV-1 Env-induced fusion repression by this tetraspanin, unlike what we see in the case of HIV-1 (Weng et al. 2009), does not require coexpression of Gag (Pique et al. 2000). At this point in time, while we do not know the mechanism of fusion regulation by tetraspanins in producer cells, it would appear plausible that these proteins do so by laterally interacting with HIV-1 Env, similar to how they are thought to organize the viral receptors in the target cell.

4 Conclusions – Perspectives

As should be obvious from my remarks above, we are only at the very beginning of understanding the mechanisms that allow tetraspanins to act at various HIV-1 replication steps. Further genetic, biochemical and cell biological analyses are clearly warranted at this point in time. Analyses of how tetraspanins regulate HIV-1 Env-triggered membrane fusion processes at the VS may also benefit from emerging knowledge about the biochemistry and the physics of cellular fusion processes. Clearly, two flat membranes opposed to each other will not

spontaneously fuse. A curved membrane however, as it exists in vesicles or at the tip of a microvillus, can get into closer contact with an opposed flat membrane (because there will be less repulsive force between the two membranes) and this will lower the energy barrier that needs to be overcome in order for membrane fusion to take place. Interestingly, expression of the tetraspanin CD9 has recently been documented to be a key requirement for the formation of proper microvilli (Runge et al. 2007). Considering also the previously mentioned finding that extracellular vesicles enriched in CD9 can trigger sperm-oocyte fusion process (Miyado et al. 2008), one is then tempted to speculate that tetraspanins act as organizers of fusion platforms not only by allowing (or not allowing) access of cellular and viral fusogens to these membrane microsegments (e.g., Singethan et al. 2008), but also by recruiting cellular proteins and lipids that promote curvature of the lipid bilayer.

In conclusion, because of their regulatory functions in fusion platforms that are situated at both sides of the VS, as well as within virions, it will not be easy to dissect exactly how tetraspanins regulate the HIV-1 transmission process. It should also be pointed out that while virus transmission in lymph nodes takes place primarily within a static setting of cells, we know very little about cell-to-cell transmission process in other organs, e.g., in the gut-associate lymphoid system. Quite likely, motile HIV-1-infected cells serve as source for the distribution of the virus at some of those other sites. It will thus eventually become imperative to study tetraspanin functions under conditions that reflect these physiological circumstances, all the more so given that tetraspanins such as CD63 (Mantegazza et al. 2004) or CD9 and CD81 (Takeda et al. 2008) also regulate cell motility, which in turn will probably influence HIV-1 transmission to target cells and thus overall virus dissemination in situ.

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