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Tetraspanin functions during HIV-1 and influenza virus replication

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Abstract

By virtue of their multiple interactions with partner proteins and due to their strong propensity to multimerize, tetraspanins create scaffolds in membranes, recruiting or excluding specific proteins needed for particular cellular processes. We and others have shown that (i) HIV-1 assembles at, and buds through, membrane areas that are enriched in tetraspanins CD9, CD63, CD81 and CD82, and (ii) the presence of these proteins at exit sites and in viral particles inhibits virus-induced membrane fusion. In the present paper, I review these findings and briefly discuss the results of our ongoing investigations that are aimed at elucidating when and how tetraspanins regulate this fusion process and how such control affects virus spreading. Finally, I give a preview of studies that we have initiated more recently and which aim to delineate exactly when CD81 functions during the replication of another enveloped RNA virus: influenza virus.

Keywords

Env; HIV-1; influenza; membrane fusion; tetraspanin; virus replication

HIV-1 assembly and release

Upon assembly, HIV-1 particles exit infected cells by budding through either the plasma membrane (in T-lymphocytes and in epithelial cells) or apparent intracellular membranes (e.g. in macrophages), at least some of which appear to be continuous with the plasma membrane (for a review, see [1]). However, viral budding does not occur all over the membrane but is restricted to relatively discrete areas. Indeed, *in vivo*, where this virus replicates primarily in secondary lymphoid organs, e.g. in lymph nodes, these areas are located at sites of cell–cell contact, and HIV-1 is thought to be transmitted very efficiently when producer and target cell align (see below). It is also well established that HIV-1 release occurs at membrane microsegments enriched in sphingomyelin, glycosphingolipids and cholesterol (reviewed in [2]), i.e. at so-called lipid rafts (for a recent review, see [3]), and that a particular phospholipid, PtdIns(4,5) P_2 , is involved in the targeting of HIV-1 release [4,5]. Furthermore, we have known for almost 20 years that this virus incorporates

specific host cell proteins, such as MHCII and the tetraspanin CD63 (for reviews, see [6,7]). More recently, our group and others have shown that not only CD63, but also other tetraspanins, are present at the viral exit site and, consequently, are acquired by the budding virions. Indeed, electron and fluorescence microscopy examination and biochemical analyses by several laboratories unequivocally showed that HIV-1 buds through TEMs (tetraspanin-enriched microdomains) containing CD9, CD63, CD81 and CD82 in macrophages, dendritic cells and T-cells (as reviewed recently in [8]). However, although their presence at exit sites suggested a role for tetraspanins in virus release, whether or not they affect HIV-1 exit remains an unresolved issue: two laboratories have published data suggesting that some members of the tetraspanin family of proteins act as positive release factors, whereas three other groups, including our own laboratory, did not see any negative effects on HIV-1 release if tetraspanins were knocked down in virus-producing cells and, to date, tetraspanins do not appear to us to be functionally involved in virus budding [8]. Nevertheless, our own data [9,10], together with those in another paper [11], also demonstrate that tetraspanins are not mere bystanders of HIV-1 egress and transmission to target cells, but that they regulate membrane fusion that is mediated by Env, the envelope glycoprotein of HIV-1, as discussed below.

Tetraspanins repress HIV-1 Env-induced membrane fusion

Although tetraspanins are considered to be organizers (*in cis*) of cellular membranes, their overall 'job description' remains relatively vague. One of the few specific functions that have been clearly established for several members of the tetraspanin family is their regulation (positive or negative) of membrane fusion processes. For example, one of the tetraspanins that we identified to be present at HIV-1 release sites, CD9, is absolutely required for sperm–egg fusion. Although it remains unknown how exactly tetraspanins control fusion processes, it is thought that they do so by manipulating the interactions of fusogenic proteins with other membrane proteins (integral or peripheral). Given these fusion regulation functions, and given that viral infections require the fusion of viral and cellular membranes, we reasoned that tetraspanins might also regulate virus-mediated fusion processes. We thus started to analyse whether overexpression (either transient or stable) or down-regulation of tetraspanins that we found localized at virus exit sites affects HIV-1 Env-induced membrane fusion. Somewhat unexpectedly, but as first reported also by Koyanagi and colleagues [11], we found that tetraspanins, when incorporated into newly formed virions, repress Env-induced virus–cell fusion [10]. Considering these findings, one could be tempted to hypothesize that tetraspanins affect HIV-1 replication negatively and that they thus restrict virus spreading. However, the mode of HIV-1 spreading may allow this virus to actually benefit from fusion repression by tetraspanins. As mentioned above, it is now thought that HIV-1 is transmitted most efficiently directly from cell-to-cell, i.e. if particles are released at cell–cell junctions, where they have immediate access to target cells [12]. Although, at first sight, such an alignment of producer and target cell may appear to just create a regular cell–cell contact zone, it should actually surprise us somewhat: one would have to expect that producer cells, which express Env at their surface, would fuse with target cells, which express CD4 and chemokine receptors; however, only rarely do the cells actually fuse. Why do they typically not form a syncytium? Adherence without fusion

may be explained at least partially by the recently documented maturation-dependence of Env fusogenicity. Unprocessed HIV-1 Gag was shown to repress Env fusion activity through an interaction with the cytoplasmic tail of Env [13,14]. 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 (see, e.g., [15]). It thus seems likely that viral and cellular proteins, including tetraspanins, act in concert to promote efficient particle transfer by repressing Env-induced membrane fusion. Indeed, when we tested whether tetraspanins also repress Env-mediated cell–cell fusion, we found that this was the case: tetraspanin overexpression in HIV-1-infected cells inhibits their fusion with target cells and thus the formation of syncytia, whereas tetraspanin ablation in producer cells augments syncytium formation [9]. Furthermore, and in line with our previous study in which we demonstrated that Env co-localization with tetraspanins is strongly enhanced by viral Gag [16], we also found that the tetraspanin-mediated regulation of Env-induced cell–cell fusion depends on Gag expression. Interestingly, the findings that tetraspanins present in HIV-1 producer cells limit virus-induced cell–cell fusion are reminiscent of results published in 2006 [17], which showed that the presence of tetraspanins in target cells also limit syncytium formation.

Chicken or egg: does the virus recruit tetraspanins to its exit site, or is viral Gag targeted to TEMs?

Irrespective of whether tetraspanins act as positive or negative factors of HIV-1 spreading, the finding that this virus exits through TEMs raised the question of whether it buds through preformed tetraspanin-rich domains, or whether these areas form only upon expression and assembly at the plasma membrane of the internal structural proteins of HIV-1, i.e. Gag. In collaboration with the laboratory of Pierre-Emmanuel Milhiet (Université de Montpellier, Centre de Biochimie Structurale, Montpellier, France), we approached this question using different high-resolution microscopy-based methods. The results of these studies clearly show that Gag assembly leads to the coalescence of small TEMs and that the trapping by assembled Gag of CD9 leads to the formation of larger membrane domains that are enriched in tetraspanins [18]. Whereas this information still does not allow us to conclude that these proteins promote HIV-1 spreading, the fact that the virus apparently not only buds through tetraspanin-rich membrane domains, but also evolved to form such membrane areas, when at the same time tetraspanins overall are down-regulated in HIV-1-infected cells [10] suggests, in our opinion, that tetraspanins are likely to be cellular factors that enhance virus dissemination.

When during the viral entry process do tetraspanins function?

In order to start to understand how tetraspanins affect the transmission of HIV-1, we have begun to delineate exactly when these proteins act to, ultimately, prevent Env-mediated virus–cell and cell–cell fusion. We are currently using various biochemical, cell biological and virological assays to determine when, relative to inhibitors against known intermediate steps in the entry process, tetraspanins act. So far, the results of these studies suggest that tetraspanins function after the initial binding (mediated by an interaction of Env and CD4), as also suggested at least for virus–cell fusion by Koyanagi and colleagues [11], but before the so-called hemifusion step (when the outer leaflets of the two membranes, but not yet the

inner ones, fuse) (M. Symeonides, J. Weng and M. Thali, unpublished work). We are also currently undertaking structure–function analyses to identify regions in both Env and tetraspanins that mediate fusion repression by the latter, and we expect that the results of these studies, together with the kinetic analyses discussed above, will give us a better understanding of how tetraspanins regulate HIV-1 replication.

Certain tetraspanins also regulate influenza virus replication: they enhance virus entry and release

Very recently, the tetraspanins CD81 and CD9 were also implicated in the influenza virus life cycle, when they were shown by Peter Palese, Megan Shaw (both at the Mount Sinai School of Medicine, New York, NY, U.S.A.) and colleagues to be incorporated into influenza virions [19]. This finding suggested a potential role for tetraspanins during the late stages of the virus life cycle. It conflicted, however, with earlier findings from our own laboratory, which indicated that, whereas HIV-1 buds from TEMs, influenza virus exits through microdomains that are not enriched in tetraspanins [20]. To address this discrepancy, reagents and techniques were exchanged and it was concluded that purified influenza virus particles do indeed contain CD9, CD81 and a minor amount of CD63. Apparently, if one analyses less pure preparations of virus produced in HeLa cells (which was done in our laboratory, where, at the time, we wanted to directly compare tetraspanins at exit sites of influenza virus with those of HIV-1, for which HeLa cells are a standard cell line for assembly studies), large amounts of HA (haemagglutinin)-positive (but tetraspanin-negative) cellular vesicles (and possibly incomplete virus particles) mask the signal from true virions. Interestingly, preliminary results from a collaborative study performed in the laboratory of Megan Shaw and our laboratory suggest that, unlike what we have seen for HIV-1, some tetraspanins not only are acquired by influenza virus, but also enhance the release of those viral particles (Y. Liang, M. Lambel, M. Thali and M. Shaw, unpublished work).

Furthermore, a recent genome-wide RNAi (RNA interference) screen performed by Megan Shaw's group identified CD81 as being required for influenza virus entry [21], suggesting that tetraspanins, like in the HIV-1 replication cycle, play roles early and late in the viral replication cycle. Together, our groups have thus started to analyse exactly how tetraspanins (positively) regulate influenza virus spreading.

Conclusions

Altogether, work over the last few years by several different groups has clearly established that tetraspanins interact with two different RNA viruses: HIV-1 and influenza virus. Also, so far, these membrane organizers appear to support (rather than limit) the spread of both viruses, although the mechanisms by which they achieve such enhancement of virus dissemination probably differ between the two viruses.

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Abbreviations used

TEM tetraspanin-enriched microdomain

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