Chapter 15
Tetraspanins as Facilitators of Viral and Cellular Information Transfer

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Abstract Cells that are infected by a virus can infect other cells by passing on the viral genome. Such transfer of viral genetic information can occur by fusion of infected and uninfected cells, or through extracellular particles that are shed from infected cells and which carry the viral genome together with viral structural proteins and/or enzymes to uninfected target cells. The transfer of viral information, either by cell-cell fusion or via viral particles, requires the coordination of numerous membrane-based functions, several of which are regulated by tetraspanins.

In many ways, viral particles resemble cell-derived vesicles such as, for example, exosomes, which are known to transport (structural, biochemical, and genetic) information through extracellular space. With this brief essay, in addition to reviewing what we currently know about various tetraspanin functions during the replication of enveloped viruses, I will also discuss some of the similarities between viral and cellular information transfer processes. Emphasis will be placed on how, in either case, tetraspanins can facilitate short- and long-range transmission as well as transfer via cell-cell fusions.

As is true for any biological function, and as so elegantly expressed almost four decades ago by the geneticist Theodosius Dobzhansky, who, referring more generally to living things, wrote that "nothing in biology makes sense except in the light of evolution" (Dobzhansky 1964), a true understanding of how viruses (here discussed initially as genetic entities, not pathogens) are propagated, and how cells communicate with each other, requires that we put things into evolutionary perspective. An introductory, more general discussion of the relationship between viruses and cells seems necessary within the context of this review article for these two

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reasons: (a) enveloped viruses obviously interact with many different cellular proteins, and an understanding of how and why viruses use tetraspanins for their successful propagation will be facilitated if we compare viral and cellular functions of these proteins; (b) the Human Genome Project and other recent sequencing efforts revealed that viruses and virus-like genetic entities have an enormous presence, and the latter are tightly intertwined with the non-viral genome, likely reflecting (as briefly discussed below) that they predate cellular genomes and indeed provided the basis for non-viral, i.e. cellular genomes (Koonin et al. 2006; Holmes 2011). Nevertheless, many biomedical researchers and most textbooks unfortunately still adhere to the idea that viruses are cellular genes that "escaped" from cells, or even that they are the remnants of formerly independent cellular microbes and thus that they constitute, in the immunological sense, "non-self" genetic entities. Acknowledging that present day viruses and cells have the same root(s), and that viruses and virus-like genetic elements, overall, more appropriately should be seen as genetic symbionts, does affect how we interpret their interactions with cells. In turn, such interpretations are likely to lead to the development of more sophisticated treatments of diseases caused by pathogenic viruses.

What, then, do we know about the origin of viruses, about their evolution? And what is known about the interaction of cells in early life?

15.1 Origin of Viruses

While there is still quite some debate about the early history of the main groups of viruses, particularly about when and how DNA viruses entered the picture (e.g. Forterre 2005), it is now generally accepted among evolutionary biologists that viruses, (again, in this introduction discussed as genetic entities, and also including so-called endogenous viruses, i.e. viruses that never leave cells), are as old as cells, or that they even predate cells (for a very recent review, e.g. Holmes 2011). This notion may seem at odds with the textbook definition of viruses, which describes them as "obligate intracellular parasites". However, the textbook definition typically is used in a context where present day viruses are described as (pathogenic) invaders of cells, and thus clearly not with an eye on their origin and thus the true nature of these genetic entities. Most biologists who study early life however, would probably agree with the notion that early viruses, or what can be considered being the ancestors of present day viruses, were RNA-based, self-replicating genetic entities. Part of these self-replicating elements, which together formed the primordial gene pool, evolved and "stabilized" to become cellular genomes (still RNA-based first, and eventually transformed into DNA-based genomes), while others evolved to become present day viruses (described e.g. in Koonin et al. 2006; Villarreal and Witzany 2010). Because the latter, i.e. the viruses, continue to use cellular resources for their replication, one can describe them as "parasitic" entities, although given their very significant presence in e.g. the human genome (see below), and given their enormous contribution to the evolution of present day species, and, perhaps, even an involvement in ontogeny (e.g. Muotri et al. 2010; Studer 2010), the term still seems too

narrow and ultimately inappropriate, as it introduces a biased view of things which is detrimental to a real understanding of the virosphere and to some extent also of pathogenic viruses.

15.2 Cell-Cell Communication

Another important aspect of our current understanding of early life is the interdependence of cells, i.e. of what are generally considered to be the basic units of life. Early cells, and also what were likely their predecessors, assemblages of primitive genetic elements, relatively freely shared genes (RNA- and later DNA-based), proteins and metabolites (e.g. see Woese 2002). While it is known for quite some time that present day bacteria still rather freely exchange genetic information, and that horizontal gene transfer (HGT) is ongoing and prevalent to the point that it is difficult to define bacterial species, only since the advent of the Human Genome Project, and the sequencing of additional, non-human genomes, are we beginning to understand that eukaryotic genomes are also far from being stable entities (Lander 2011). We now know that about half of the human genome consists of virus-like (often repetitive) sequences, and while some of these may be the result of infections by exogenous viruses that have crossed the species barrier at some earlier point in time, the majority of them most likely are remnants and expansions of the early genetic make-up of cells, as discussed above. Even more importantly, though we are only at the very beginning of understanding their complexity, it is already clear that these and other sequences, including non-coding genetic elements, are responsible for the evolution of novel classes of animals, or of specific species, including humans (Lander 2011). Viviparous mammals, for example, are thought to have evolved as a consequence of the infection of germ line cells of pre-mammals by retroviruses, whose envelope glycoproteins (which can trigger the fusion of plasma membranes of adjacent cells) were subsequently adapted by cells for the formation of syncytiotrophoblast, and thus placentas (e.g. Mi et al. 2000).

Finally, and directly relevant for the discussion of specific tetraspanin functions in this chapter, it is becoming clear that the flux of genetic information is not restricted to the evolutionary timescale, or to HGT between bacteria, but that transfer of genetic (and of course also biochemical) information also takes place within multicellular organisms, and between the myriad symbionts that cohabit multicellular organisms (for a further discussion of this, e.g. see (Goldenfeld and Woese 2007), also see below).

15.3 Tetraspanins and Other Scaffold Proteins as Mediators of Information Transfer

Cellular membranes are central to the above-described exchange of information between cells, whether viral or cellular in nature. Also, in order to process, i.e. transmit, biochemical and genetic information properly, membrane-based regulatory

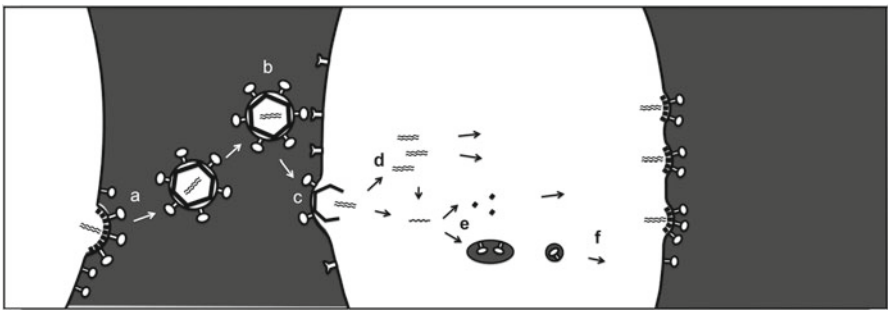

Fig. 15.1 Transmission and multiplication of an enveloped virus. Tetraspanins have been implicated in the regulation of viral assembly and release (**a**), particle attachment (**b**) and entry (**c**), genome replication (**d**) and expression (**e**), and transport (**f**) of viral components

units had to evolve, and it appears reasonable to assume that families of scaffold proteins, such as tetraspanins, did indeed evolve for that reason. While several chapters in this volume review their function, i.e. describe mechanism by which distinct members of the tetraspanin family of proteins control specific processes, in the remainder of this chapter I will discuss how some of these cellular regulatory activities are also used to control the transmission of viral genetic information. However, our understanding of how tetraspanins function during the replication of various viruses, with the exception of HCV (see the chapter by Cocquerel and Silvie), is still very much in its infancy, and we are thus only starting to comprehend similarities between tetraspanin activities that regulate cellular functions and the role of these proteins in life cycles of various viruses (Martin et al. 2005; van Spriel and Figdor 2010; Thali 2009, 2011). In Fig. 15.1, I provide a scheme for the transmission event and replication cycle of a generic enveloped virus. The figure illustrates that tetraspanins have functions at different steps of viral life cycles. Indeed, this multifunctionality complicates the analysis of their roles to some extent, as e.g. ablation of a specific tetraspanin can at the same time lead to repression of one step but enhancement of another step in the viral replication, resulting in unclear phenotypes.

15.4 Long-Range Information Transfer Via Viral Particles, Exosomes and Other Cellular Microvesicles

Decades ago, and thus long before results of the Human Genome Project told us that viruses and virus-like genetic entities make up a very significant fraction of the genome of cells, Baltimore, Campbell, Darnell, and Luria in the last paragraph of the textbook General Virology (Luria et al. 1978), declared: “A virus is essentially part of a cell”. Despite this (in retrospect) visionary statement, many scientists, including

many virologists, think of viruses primarily as entities that exist outside of cells, i.e. of particles. Therefore, I will first discuss what we know about the involvement of tetraspanins in the transport of viral information (genes, proteins) via extracellular particles.

15.4.1 Tetraspanins and Release of Viral Particles

The first virus that was shown to be associated with a tetraspanin was HIV-1. Almost two decades ago, two groups reported that HIV-1 particles are enriched in CD63 (Meerloo et al. 1992, 1993; Orentas and Hildreth 1993), and numerous additional reports have since confirmed that finding (e.g. see (Chertova et al. 2006), reviewed in (Thali 2009)). Though it was demonstrated that the incorporation was specific, only recently several groups tested if this tetraspanin, and some other members of the family (CD9, CD81 and CD82) which are also acquired by virions, play a functional role in the formation of HIV-1 particles. Conflicting results have been reported about CD81, with one group showing a positive role for CD81 in the release of virus from Molt T cells, but two other groups not finding evidence for HIV-1 particle release enhancement by this tetraspanin in 293T cells or in Jurkat T cells (Sato et al. 2008; Krementsov et al. 2009; Grigorov et al. 2009). Further, none of the studies that measured if altering of CD63 levels affected virus particle release found a positive effect of that tetraspanin (Sato et al. 2008; Krementsov et al. 2009; Ruiz-Mateos et al. 2008). Similarly, despite the presence of CD9 at exit sites of another retrovirus, feline immunodeficiency virus (FIV) (de Parseval et al. 1997, and references therein) and at the release site of canine distemper virus (CDV) (Singethan et al. 2008), (Loffler et al. 1997) this tetraspanin also does not appear to influence release of viral particles (Schneider-Schaulies and Thali, unpublished observations). This latest observation regarding FIV was particularly surprising, as the paper by Elder and colleagues (de Parseval et al. 1997) very clearly documented that the treatment of FIV-producing cells with an anti-CD9 mAb resulted in reduced amounts of released virus, something that was subsequently confirmed by us in a study in which we also showed inhibition of HIV-1 release by another anti-CD9 antibody (Khurana et al. 2007). More recent work however revealed that the antibodies used in those two studies (Vpg15 and K41, respectively) were unique, as they both cluster CD9 and other associated tetraspanins at the plasma membrane of closely aligned cells. This may have lead to an unspecific interference with the viral budding process and/or a trapping of viral particles in a dense network of newly formed microvilli (which we called “microvilli zippers”) at the cell-cell junction (Singethan et al. 2008). (Indeed, further studies of how binding of K41 to CD9 leads to the formation of microvilli may help revealing the mechanisms of microvilli formation per se, a process in which CD9 is known to play a key role (Runge et al. 2007)).

In contrast to the lack of support by tetraspanins of retroviral release (with the exception of the reported CD81-induced enhancement of HIV-1 shedding from certain T cells, see (Grigorov et al. 2009)), and as revealed by more recent investigations,

members of the tetraspanin family may function as promoters of particle release for two other viruses: Tetraspanin 7, through an interaction with the viral capsid protein HP26, was recently shown to enhance the release of herpes simplex virus type 1 (HSV-1) (Wang et al. 2010), and CD9 and CD81 have been shown to be specifically incorporated into influenza virus particles (Shaw et al. 2008), suggesting the possibility that these tetraspanins promote particle shedding. In both cases, the (potential) mechanisms by which these tetraspanins (might) enhance particle release have yet to be elucidated. Further, a very recent report (Verweij et al. 2011) suggests that the tetraspanin CD63 can regulate the release of a viral component: while not addressing yet molecular mechanisms either, it documents that the viral oncogene latent membrane protein 1 (LMP1) of Epstein Barr virus associates with CD63, thus allowing LMP1 to be secreted via exosomes. This downregulation of LMP1 (due to its CD63-mediated secretion) leads to diminished NF-κB-induced cell activation, which is thought to favor virus persistence.

15.4.2 Tetraspanins in Viral Particles Can Modify Their Infectivity

Given that some tetraspanins are clearly enriched in HIV-1 particles, yet do not appear to play a role in release of that virus, several groups have asked if their presence makes the virions more infectious, e.g. by increasing their fusogenicity. So far results do not support this idea: indeed, the opposite is true, as overexpression of tetraspanins in producer cells and thus their enhanced incorporation into HIV-1 particles, while not affecting particle binding to target cells (Sato et al. 2008), renders them less infectious. Conversely, ablation of tetraspanins and thus reduced amounts of particle-associated tetraspanins correlates with increased infectivity (Sato et al. 2008; Krementsov et al. 2009). The situation might be different though for influenza virus: as discussed above, this virus specifically incorporates CD9 and CD81, and given that these tetraspanins promote oocyte-spermatozoa fusion (see the chapter by Boucheix), they might also play positive roles in the entry process of influenza virus.

15.4.3 Tetraspanins in Exosomes

Interestingly, shortly after we learned about the presence of tetraspanins in virions, it was also reported that exosomes are enriched in these scaffold proteins (Escola et al. 1998). Exosomes are extracellular vesicles of 30–100 nm that are secreted by most of the cells. Biogenesis of exosomes involves membrane budding into early/late endosomes, thus forming multivesicular bodies or multivesicular endosomes (MVBs/MVEs). Upon fusion of MVBs/MVEs with the plasma membrane, exosomes are shed into extracellular space (for an early review of these vesicles, particularly of their composition, see (Thery et al. 2002), see also the chapter by Ashman and Zoller for more information on exosome functions). At first sight, viral

particles and exosomes, besides their acquisition of tetraspanins, do not appear to have much in common, indeed even their sizes do not really match (Pelchen-Matthews et al. 2004). Nevertheless, probably also because both use the same machinery for their formation (e.g. see Morita and Sundquist 2004), it was hypothesized that, at least functionally, exosomes and HIV-1 could be related (Nguyen et al. 2003; Gould et al. 2003), and more recent reports showed that, like viruses, exosomes can transport genetic information (in the form of mRNAs and microRNAs) (e.g. see Valadi et al. 2007). Further, and even more impressively, the glycome of HIV-1 released from T cells is identical to that of exosomes released from the same cells (Valadi et al. 2007; Krishnamoorthy et al. 2009), lending very strong support to the idea that viruses and exosomes can use the same cellular pathways for their generation. Exosomes are not the only microvesicles that can be produced by cells. Indeed we learn more and more about transfer of material or signals from cell-to-cell via small vesicles (e.g. Mack et al. 2000; Gillette et al. 2009), and it is already well established that some of these vesicles play important roles in various physiological processes (e.g. Cocucci et al. 2009). Importantly, such vesicles, whether they carry mRNA and/or microRNAs or merely proteins, can clearly modify the behavior of cells that are distant from the cell that released the vesicles, and in that regard they are biological entities that behave like viral particles.

What roles do tetraspanins play in information transfer via exosomes? To the best of our knowledge, no mechanistic studies have addressed that question yet. However, given that some tetraspanins can promote the release of certain viral particles, as discussed above, it would seem conceivable that they also can enhance exosome formation. Indeed, CD9 and CD82 expression was recently shown to augment the release of β-catenin-containing exosomes (Chairoungdua et al. 2010). Interestingly, like the above-mentioned CD63-induced release of EBV's LMP1, such enhancement of β-catenin-containing exosome release by CD9 and CD82 leads to reduced cell signaling. Do tetraspanins also play a role once they have been incorporated into exosomes? Based on the fact that tetraspanin incorporation can render viral particles less fusogenic and thus can inhibit their entry into target cells, it would appear likely that they can also regulate exosome-plasma membrane fusion processes. Indeed in one case, they are known to do that: for exosomes that are released from eggs before fertilization, a member of the tetraspanin family (CD9) was shown to act as a positive fusion regulator, as will be briefly discussed below (Sect. 15.6) because of similarities to virus-induced cell-cell fusion.

15.4.4 Tetraspanins and Virus Entry into Cells

So far I have discussed roles that tetraspanins play (or not) in the release of viral particles and cellular membrane vesicles, and their role in mediating the release of the viral genome into target cells. How about tetraspanins that are present at the surface of the target cells, do they have functions there as well, similar to e.g. how one of them (CD81) acts as co-receptor for HCV (see the chapter by Cocquerel and

Silvie)? While no information is available yet regarding attachment and entry of cellular vesicles, such as exosomes, several studies have shown that tetraspanins on target cells regulate virus attachment and entry. Two independent studies have suggested that CD63 and other tetraspanins can enhance HIV-1 uptake by macrophages (von Lindern et al. 2003; Ho et al. 2006), whereas one other study showed that their presence at the surface of T lymphocytes prevents fusion of viral and (T) cellular membranes (Gordon-Alonso et al. 2006). While nothing is known yet about how the presence of tetraspanins enhances virus uptake into macrophages, data presented in a recent study indirectly suggest that they could do so e.g. by negatively regulating virus-T cell fusions by re-organizing the receptors for HIV-1, i.e. CD4 and chemokine receptors (Barrero-Villar et al. 2009). Also, CD63 is engaged in the trafficking of CXCR4, one of the co-receptors for HIV-1, to the cell surface, and may thus co-regulate its surface levels and thus the permissiveness for virus entry (Yoshida et al. 2008). Perhaps comparably, CD63 and CD151 have been implicated in organizing the entry site for human papillomavirus (HPV) (Spoden et al. 2008), and a recent siRNA screen revealed that the presence of CD81 at the surface of target cells is critical for influenza virus entry steps (Konig et al. 2010). It should be pointed out though that in neither of these two cases (HPV and influenza virus) do tetraspanins act as co-receptors for the respective viruses.

15.5 Short-Range Information Transfer at Immunological and Virological Synapses

Cellular microvesicles, including exosomes, by using the vasculature as gateway, can travel long distances within an organism. This means that, in principal, just like viruses, certain microvesicles can even be transferred from one organism to another. However, as far as we know, many of them do not travel far at all, again resembling viral particles (as will be discussed below). Rather, they deliver their message (in the form of proteins or nucleic acids) to cells in the near vicinity of cells releasing them.

Interestingly, and as first documented in a seminal study almost three decades ago (Rodriguez-Boulan et al. 1983), it appears as if viruses fall into either one of two categories: some viruses release their newly formed particles at surface areas where the producer cell contacts a potential target cell, while others seem to be released almost exclusively at free surfaces. Delivery to target cells that are aligned with the producer cell appears to be a major route for the transmission of the human retroviruses HTLV-1 and HIV-1 and indeed for many other viruses that cause systemic infections of the host, such as HSV, measles virus etc. Research over the past two decades has established that these viruses are released from infected (and thus virus producing) cells at specialized cell-cell surface areas. Because these sites are used for the transmission of information, and also because of certain structural similarities with different types of synapses, relatively recently they have been dubbed "virological synapse" (VS) (e.g. (Jolly et al. 2004)). At the VS, the newly released

viral particles need to travel only a few nanometers, if that, before reaching the surface of the uninfected (target) cell. While this transfer mode is very efficient, it is not without risk for both the producer and the target cell: the presence of the viral envelope glycoprotein on the pre-synaptic plasma membrane together with the presence of the viral receptors on the post-synaptic plasma membrane, in principal would allow the two cells to fuse with each other. Such fusion of producer and target cells, except for under certain circumstances (see below), would probably be detrimental to virus spread because it leads to the formation of so-called syncytia. Syncytia, however, while being able to produce progeny virus, are relatively short-lived entities and can thus be viewed as dead-ends for virus dissemination. Various tetraspanins have been shown to inhibit fusion (for a recent review, see (Fanaei et al. 2011), also, see the chapter by Hemler) and it thus appears likely that viruses such as HTLV-1 and HIV-1 evolved to recruit them to the viral pre-and post synapse for exactly that reason, i.e. because they inhibit cell-cell fusion (Weng et al. 2009). I write "evolved to recruit" because recent data by our group (Krementsov et al. 2011) and also by Ono and colleagues (Hogue et al. 2011), demonstrate the non-randomness of this process: HIV-1 Gag multimerization, which precedes viral budding, leads to a trapping of various tetraspanins at the future viral exit site, thus, tetraspanin accumulation at the pre-synapse is orchestrated by viral components. Such clustering of tetraspanins at the budding site comes at a cost, however. As mentioned above, tetraspanin incorporation into newly formed viral particles also renders them less fusogenic. However, given that those newly formed particles, if released at the VS, are placed right next to the target cell, even a reduced fusogenicity is likely enough to secure infection of the target cells and thus continued spread of the virus. In that regard it is interesting to see that the aforementioned transfer of certain cellular vesicles (Gillette et al. 2009) takes place also at sites that are enriched in CD63: perhaps the presence of this tetraspanin also allows the cells to separate (without fusion) upon vesicle transfer.

Tetraspanins are recruited not only to the viral budding site, i.e. to the viral pre-synapse, but, as mentioned above, they are also present at the target cell surface, where they may associate with viral receptors (e.g. CD4, a receptor for HIV-1, Imai et al. 1995). Similar to their anti-fusogenic function at the pre-synapse, receptor-associated tetraspanins may inhibit membrane fusions from taking place (likely by different mechanisms). While such fusion prevention precludes the entry of some particles, as mentioned before, given the intimate physical association with the producer cell, this will probably not hinder the virus from infecting the cell. Indeed, just as what was hypothesized regarding tetraspanins at the pre-synapse, we reason that their presence at the post-synapse also ultimately supports virus transmission from cell-to-cell and thus virus spread. It seems even plausible that the distribution of tetraspanins at the surface of both producer and the target cell, i.e. at the pre- and the post-synapse, is fine-tuned such that they can function primarily as inhibitors of cell-cell fusion, while not interfering too much with the fusion of viral and cellular membranes. Such a sophisticated arrangement of synapse molecules is not without precedent: neural synapses clearly are spatially organized (e.g. Gerrow and El-Husseini 2006), and research over the past decade has shown an equally sophisticated

organization of yet another synapse, the so-called immune synapse (IS). In the following I will briefly discuss the relationship between the IS and the VS, also because of the similarities between the IS and the VS formed between uninfected cells and cell infected by primate retroviruses such as HIV-1 and HTLV-1 (for a recent review, see also e.g. Sattentau 2008).

ISs are formed between antigen-presenting cells (APCs) and T lymphocytes as well as between killer T cells and their target cells (which in principal can include also other T cells). Likely because APC-T cell synapses are better characterized, VSs are typically compared with these synapses rather than killer-target cell synapses, even when virus transmission between infected and uninfected T cells are described. What does the (T cell-T cell) VS have in common with the ISs formed between APC and T cells? Evidently the composition of the target cell surface is at least partially the same: the post-synaptic plasma membrane contains T cell-specific membrane proteins including CD4 and certain chemokine receptors as well as adhesion molecules such as the lymphocyte function-associated antigen 1 (LFA1). In addition to these factors, which interact with partner molecules in trans, i.e. receptors/ligands situated at the pre-synapse, the synapses include tetraspanins (e.g. CD81) which were reported to act as co-organizers. For example, studies have shown that CD81, which can act as a co-stimulator in T cells (see Levy and Shoham 2005 for a review), was shown to be redistributed during antigen presentation (e.g. (Mittelbrunn et al. 2002)). That co-stimulatory potential of CD81 may also bear fruit at the VS: it has been demonstrated that treatment of HIV-1-infected T cells with an anti-CD81 antibody leads to enhanced expression of the integrated viral genome (Tardif and Tremblay 2005). In addition, and as mentioned above, it appears likely that this tetraspanin, and possibly other members of the family, are involved in the reorganization of CD4, thus co-regulating viral entry at the post-synapse (Gordon-Alonso et al. 2006).—At first sight, the pre-synapses of the APC-T cell IS and the VS have less in common than the post-synapses. At the viral pre-synapse there are no peptide-loaded MHC complexes that would trigger an activation of the post-synaptic T cell (via cognate T cell receptors). Rather, the newly produced envelope glycoproteins at the pre-synapse engage, in the case of HIV-1, CD4 and a member of the chemokine family, e.g. CXCR4 or CCR5, and in some cases also α4β7 integrin (Arthos et al. 2008), leading to the above mentioned activation events in the post-synaptic T cell. Two other cellular elements in the pre-synaptic cell play active roles in the formation of the VS: the kinase ZAP-70 and the microtubule-organizing center (MTOC). In the APC-T cell IS, ZAP-70-induced signaling in the post-synaptic cell promotes cytoskeleton and MTOC reorganization, and T cell activation, while ZAP-70-induced cytoskeleton reorganization and MTOC polarization (in the virus-producing cell) towards the pre-synapse of the VS has been reported to be necessary for efficient cell-to-cell transmission of HIV-1 (Sol-Foulon et al. 2007). It is possible, though remains to be investigated, that tetraspanins act as co-regulators of ZAP-70 initiated events at the pre-synapse. If so, this will require that tetraspanins be properly distributed at the pre-synapse. Arguably the most important result of numerous studies of the IS over the past decade is that this synapse has a sophisticated architecture, that it is subdivided into discrete zones where certain

activities are initiated (or terminated). Further, and also of direct relevance to the VS, the synapse undergoes changes over time (Fooksman et al. 2010), and future studies of the VS, and of the role that tetraspanins play in its formation, thus should take such dynamics into account.

15.6 Information Transfer Via Fusion of (Virus-Infected and/or Uninfected) Cells

I should start this final section by pointing out that the term "uninfected cell" in this subtitle is an obvious misnomer, given that, as described above, all cells carry virus-like genetic elements that are remnants of pre-cellular life forms, and/or because at some point in time they have been infected by exogenous viruses, which, even if they are no longer active, still contribute to the overall genetic inventory of the cells. Perhaps a more appropriate term perhaps would thus be "not newly infected cell"—however, for obvious practical reasons I will continue to use the term "uninfected cells".

Uninfected cells can receive viral genetic information either when a viral particle binds to it, and releases the viral genome into the cell, or, and this arguably is the most straightforward way, when they fuse with infected cells. Fusion of virus-infected and uninfected cells leads to the formation of so-called syncytia. As mentioned above however, such syncytia formation, for many viruses, is not desirable, as syncytia are relatively short-lived entities. Nevertheless, under certain circumstances they may help spreading the virus, as they are motile and may exert force that allows them invade spaces that are not accessible to single infected cells (e.g. see Sylwester et al. 1998). And since, upon infection, i.e. here now upon cell-cell fusion, they acquire the properties of the "infecting cell", i.e. the fusion partner, as part of the new entity, the syncytia, can produce and shed progeny virus, which, again, makes them potentially useful agents of virus dissemination within their limited lifetime.

Because syncytia, under most circumstances, are detrimental to efficient spread of viruses such as HIV-1, this virus has evolved several mechanisms that tightly control the fusogenicity of its envelope glycoprotein (see Thali 2011, for a detailed discussion), and as discussed above, the recruitment of tetraspanins to the virus release site is one of them. As also discussed above, this negative regulation of membrane fusion processes is an important function of these proteins. Tetraspanins, however, can also do the opposite, i.e. they can promote membrane fusion. The most prominent case is that of CD9, whose expression in oocytes is absolutely required for fusion with spermatozoa to take place (see the chapter by Boucheix). However, it is important to point out that CD9 (and probably all the other members of the tetraspanin family) is not a fusogen itself, i.e. it does not trigger the fusion of membranes. Rather, this tetraspanin is thought to organize other membrane proteins, including cellular fusogens that mediate the fusion of the oocyte and the spermatozoa (Ziyyat et al. 2006). Because of the limited availability of human research material necessary for investigations of this particular fusion process, our knowledge of the

molecular details is not yet very advanced. We know much more about how CD81, a closely related tetraspanin, which, through its interactions with specific other membrane proteins, promotes the fusion of the hepatitis C viral (HCV) membrane with the target cell membrane (see chapter by Cocquerel and Silvie). What we do know though is potentially interesting (Miyado et al. 2008), though remains unconfirmed so far (Gupta et al. 2009): to promote sperm-egg fusion, CD9 reportedly does not need to be present at the surface of oocytes, but can be released on exosomes (Miyado et al. 2008), the cellular membrane vesicles discussed above. How CD9 on exosomes could ultimately promote sperm-egg fusion remains to be seen. Such exosomes could form bridges between sperm and egg. Because of the more curved surfaces of exosomes (relative to the surfaces of sperm and egg), the energy barrier that needs to be overcome by a fusogen to trigger mixing of two opposing lipid bilayers is lower. Such a situation is reminiscent of how viral particles, when incubated with uninfected cells, can form bridges and induce the fusion of two uninfected cells (a phenomenon called "fusion from without" (Bratt and Gallaher 1969)). It has also been suggested, however, that the exosomes may deliver CD9 to the sperm, where they could reorganize the surface of those cells such that the fusogen there may be activated (Barraud-Lange et al. 2007). Such delivery to other cells of molecules involved in the fusion process would be reminiscent how exosomes have been shown to transfer CCR5, one of the co-receptors for HIV-1 to cells, thus rendering them infectable by HIV-1 (Mack et al. 2000).

Finally, it should be pointed out that information transfer by cell-cell fusion, whether virus-induced or virus-independent, is probably an underappreciated phenomenon, and thus that tetraspanins are likely to play important regulatory roles that have not yet been detected. Besides sperm-egg fusion, and e.g. the fusion of myoblasts, there are probably many more cell-cell fusions taking place, during ontogeny and also later on (for example, see Ying et al. 2002). However, as fused cells can divide again, in many instances we may not realize that they previously received genetic information from another cell (their fusion partner).

15.7 Perspectives

Research on tetraspanin functions in virus replication, with a few exceptions, is still very much in its infancy. Nevertheless, as outlined in this chapter, these membrane proteins are clearly involved in numerous steps of virus spread. Exogenous viruses, in contrast to endogenous viruses, can function as semi-independent entities, and because of this, many fundamental principals of cellular and molecular biology were revealed through the study of these genetic entities. Given that the transmission of enveloped viruses includes, and is controlled by, numerous membrane-based processes, and because these processes, such as fusion of lipid bilayers, signaling, etc., are of obvious importance also for cellular functions, it can be expected that further analyses of tetraspanin functions in virus replication will continue to shed light also on important cellular mechanisms.

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