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SNAREs in HIV-1 assembly

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Viruses have a limited number of genes but a complex life cycle and have evolved to utilize numerous host factors to complete their replication. The assembly and budding process of enveloped viruses utilizes numerous cellular factors to facilitate transport from one membrane bound compartment to the other. The host SNARE proteins are widely involved in late stages of vesicular mediated transport by catalyzing the docking and fusion of apposing membranes in the vesicle and target compartment. By generalized disruption of the SNARE sorting machinery, we recently demonstrated a role for these proteins in HIV-1 assembly by affecting Gag localization to the plasma membrane. Whether the observed phenomenon is specifically due to SNARE disruption or generalized disturbance of the cell sorting machinery and the involvement of specific "v" vs. "t" SNAREs in this phenomenon remains to be elucidated.

Keywords: HIV-1, Gag, virus assembly, budding, SNAREs, NSF, dominant negative

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Membrane bound vesicles transport various proteins, lipids and other luminal contents to different compartments in the cell. The process of vesicular transport consists of several steps including vesicle formation, targeting to the appropriate compartment and fusion of the vesicle with the target membrane. This orchestrated movement of vesicles in the cells ensures appropriate targeting of proteins to the intracellular and extracellular compartments. The process of fusion of the vesicle with the target membrane is catalyzed by a group of proteins collectively referred to as the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) proteins.[1] Besides their role in retrograde and anterograde transport via the secretory and endocytic pathway, SNARE proteins are also involved in daughter cell separation (known as abscission) during cell division.[1-6]

The SNARE proteins are broadly classified into two main categories: the v-SNAREs that are primarily found on the transport vesicle and the t-SNAREs that associate with the target membrane.[3,6] Besides being structurally distinct, the v-and t-SNAREs perform different functions. Interactions between specific v-SNAREs on the vesicular compartment and t-SNAREs on the target membrane leads to the formation of the trans-SNARE complex which upon fusion of the lipid bilayers is converted into a cis-SNARE complex. Upon cargo delivery, the cis-SNARE complex is disassembled by the concerted efforts of the ATPase N-ethylmaleimide (NEM)-sensitive factor (NSF) and α-soluble NSF attachment protein (α-SNAP).[3,6] Dominant negative mutants of NSF like K266A and E329Q lead to defects in disassembly of cis-SNARE complexes resulting in generalized disruption of SNARE function.[7]

Viruses being simple organisms carry the minimal genes essential for replication in the host and make use of the cellular machinery for completion of their life cycle. Since the SNARE proteins are universally important for vesicle-mediated transport, it is tempting to speculate that they may play a role in HIV protein transport, virus assembly and/or budding. Furthermore, the final stages of HIV budding, specifically the release of virions from the cell surface, shows marked resemblance to the process of abscission[8,9] suggesting that both phenomena may be catalyzed by similar factors. With this

premise in mind we studied the role of SNARE proteins in the HIV life cycle using the ATPase-defective NSF mutant E329Q that prevents disassembly of the cis-SNARE complexes thereby resulting in a general disruption of the SNARE machinery.[10] Using this method of inhibition of the SNARE sorting machinery we recently demonstrated that they play a role in HIV assembly/release.

Upon dominant-negative NSF expression, we not only saw defects in Gag localization to the plasma membrane but also observed defects in CD63 and CD81 localization to membranes. Moreover, localization of other membrane receptors like CD4, CXCR4 and CCR5 to the plasma membrane was also affected.[10] Considering the role of SNARE proteins both in cell division and in intracellular vesicle-mediated transport events and membrane fusion,[1-6] we had anticipated that SNARE disruption may lead to virion tethering to the plasma membrane or virion-virion tethering, defects similar to those caused by Tsg101 depletion.[11] On the contrary, we found that SNARE disruption led to defects in an earlier step—plasma membrane association of HIV-1 Gag. Moreover, only the membrane-bound Gag (whether bound to plasma membrane or internal membrane) was affected by SNARE disruption. Our findings suggest that SNARE disruption either directly or indirectly plays a role in Gag binding and/or transport to the plasma membrane, which in turn leads to defects in virus release.

The minimum viral domain required for retrovirus budding is the Gag polyprotein, expression of which is sufficient to drive the formation of virus-like particles (VLPs).[12] The HIV-1 Gag polyprotein is synthesized in the cytosol following which it rapidly translocates to the site of virus assembly.[12,13] Although the plasma membrane is believed to be the predominant site for HIV-1 assembly, the itinerary that Gag follows to get to this site remains much disputed.[14-16] Our study suggests that Gag may traffic with membrane-bound vesicles incorporating SNARE proteins that are targeted to the plasma membrane. It is also possible that these Gag-laden vesicles are first taken to intracellular compartments like the late endosomes/multi-vesicular bodies (MVBs) that may also serve as sites of virus assembly.

The fact that SNAREs function not only in the secretory but also the endocytic pathway[17] suggests that depletion of the SNARE function would result in an overall defect in intracellular membrane compartments. DN NSF used in our study prevents disassembly of the cis-SNARE complex and recycling of the proteins, so the function of SNARE proteins would be unidirectional with the accumulation of cis-SNARE complexes on membranes.[18-20] Thus, an overall disruption of intracellular membrane compartments would be anticipated. With the assumption that Gag associates with intracellular membrane bound-compartments, a deficiency of these would ultimately lead to the cytosolic pattern of Gag localization. This was consistent with our observation that CD63/CD81, markers of late endosomes,[21-23] were also rendered cytosolic upon SNARE disruption.

SNARE proteins are involved in the transport of not only proteins but also the majority of lipids between membrane compartments. A disruption of SNARE function would also result in dysregulation of the lipid components of various membrane compartments in cells. One such biologically active lipid phosphatidylinositol(4,5)bisphosphate [$PI(4,5)P_2$], has been shown to be critical for HIV budding by promoting Gag binding to plasma membrane via interaction with the basic residues in the matrix domain.[24-27] Whether disruption of SNAREs leads to a deficiency of $PI(4,5)P_2$ on the plasma membrane and subsequently Gag binding remains a possibility needing further investigation. In the same context, it has been shown that HIV buds via distinct lipid raft-enriched microdomains[28,29] in the plasma membrane. Whether SNARE disruption affects raft formation/function also needs to be examined.

There is a great deal of redundancy in the function of SNARE proteins, as different SNARE members can give rise to multiple SNARE complexes. Moreover, a given SNARE can not only be incorporated into different SNARE complexes but the same t-SNARE can interact with different v-SNAREs and mediate varied transport events in different cell types. For example, VAMP-3 can functionally replace VAMP-2 and several different SNARE complexes are functional in different cell compartments like ER, golgi, endosomes, plasma membrane, etc.[3,6] It will be important to determine whether specific v-or t-SNAREs play a more important role in budding than others. Considering the significant redundancy in SNARE protein function, it is likely that disruption of individual v-or t-SNAREs would not have an effect on virus budding but the combined disruption of multiple SNAREs may affect assembly/release. It would also be interesting to test whether SNARE disruption affects HIV assembly in physiologically relevant cell types like T cells or macrophages. Moreover, it also needs to be investigated whether SNAREs are important only for retrovirus release or whether other diverse intracellular pathogens also make use of the SNARE machinery for budding.[30] Considering the fairly conserved nature of these proteins, we speculate that they may be important for other intracellular pathogens as well.

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