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Real-Time Imaging Reveals that HIV-1 Vpr Dissociates from the Core and Accumulates in the Nucleus after Viral Fusion

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Viral protein R (Vpr) is an HIV-1 accessory protein that associates with capsids during viral assembly and is important for infections in non-dividing cells. Vpr functions in host cells by inducing G2 cell-cycle arrest, and regulation of cellular proliferation and apoptosis. Vpr has two nuclear localization sequences that direct its transport to the nucleus. Fluorescently labeled Vpr (YFP-Vpr) is widely used to visualize HIV-1 cores in the cytoplasm during entry. Here we report on the dissociation of YFP-Vpr from HIV-1 cores shortly after viral fusion and its subsequent accumulation in the nucleus. Real-time live cell imaging showed that, under conditions of productive entry and infection, Vpr dissociated from cores post-fusion and accumulated in nuclei over time scales that correlated with the kinetics of viral fusion (1/2∼15 min). Nuclear accumulation of Vpr scaled with the number of cell-bound virions and could be blocked by lysosomotropic agents or a fusion-inhibitory peptide. These effects were observed in two cell lines and were independent of the fusion proteins incorporated into viral particles. Fluorescence recovery after photobleaching of YFP-Vpr within the nucleus revealed quick (1/2∼3 min) recovery, indicating how dissociation from capsids is a rate-limiting step in Vpr post-fusion transport. Fluorescence correlation spectroscopy measurements on post-fusion nuclear YFP-Vpr, yielded fast and slow diffusive components (D∼10 μm²/s and 0.8 μm²/s, respectively) similar to those measured for YFP-Vpr over-expressed in cells. These diffusion coefficients reflect that nuclear Vpr exists in two forms - as a monomer, and in large complexes with host proteins or perhaps even chromatin structures. Current efforts are underway to explore the determinants of the stability of Vpr-capsid complexes. This work was supported by the NIH R01 GM054787 grant.

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Delivery of Liposomal Contents to Outer Membrane Vesicles from Gram Negative Bacteria

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Gram negative bacteria produce small ∼50-200 nm vesicles from their outer membranes. These outer membrane vesicles (OMV) have been implicated in activities such as transmission of virulence factors, horizontal gene transfer and development of biofilms. In this investigation, we continue our studies on the association and/or fusion of various liposomes with OMV. The delivery of large encapsulated molecules into OMV from L. enzymogenes C3 was investigated using liposomes with lipid compositions previously observed to be apparently fusogenic (Bartos et al., Biophys. J. 104(2) suppl1, 90a). Liposomes (100 nm in diameter composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) in a 0.3 ratio or a palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) were used to encapsulate dextran conjugates of Texas red averaging 40 kDa. They were incubated with Lysobacter OMV (30°C, 1 hr.), then sedimented through 15% iodixanol, and the fluorescence monitored as indicative of transfer of liposomal contents to fused products. Both liposomal compositions showed significant evidence of dextran transfer. Because biofilms also contain OMV, the interaction of these liposomes with E. coli (DH10B) biofilms was also investigated via fluorescence microscopy. Significant penetration and binding within the biofilm mass was observed, as well as possible fusion with OMV, and rarely, evidence of transfer of dextran into whole bacterial cells. Fluorescence resonance energy transfer (FRET)-based assays also demonstrated that liposomes as small as 30 nm could rapidly fuse with Lysobacter OMV suggesting possible delivery to OMV with smaller perturbation and better biofilm penetration.

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Fusion Fore Dilution by Snare Proteins

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Fusion fore dilution describes the release of fusion active and inactive fusogen molecules during fusion progress, which can affect the rate of fusion. We use the yeast vacuole fusion system to study fusion fore dilution. We first show that a series of mutation in yeast SNAREs leads to increased fusion fore dilution. Moreover, we find that the fusion fore dilution increases with increasing fusion rate. We also show that the fusion fore dilution can be decreased by increasing the rate of vesicle delivery. Our results suggest that fusion fore dilution is a general feature of fusion, and it may play a role in the regulation of fusion rate.

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Control of Fusion of Pore Nucleation and Dynamics by SNARE Protein Transmembrane Domains

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Membrane fusion is a fundamental biological process, whose initial stages have been observed in hormone-secreting cells and neurons using electrophysiological and biochemical methods. The initial connection between the plasma membrane and a hormone- or neurotransmitter-filled vesicle - the fusion pore - can flicker open and closed repeatedly before dilating or rescaling irreversibly. Pore dynamics determine events such as vesicle recycling and release kinetics, but pore properties are poorly known, because fusion pores are transient, and biochemically defined assays with single-pore sensitivity are lacking. We isolated single flickering pores connecting v-SNARE-reconstituted nanodiscs to cells ectopically expressing cognate, “flipped” t-SNAREs. Using newly developed, large NDs that are 21-27 nm in diameter, we varied v-SNARE copy numbers from zero to up to 11 per ND face. Pore nucleation required a minimum of 2, and reached a maximum above ~4 copies per face, but the probability of pore dilation was far from saturating at 11 copies, the maximum that the NDs could hold per face. Our results indicate that copy numbers of available SNAREs may be pivotal in determining whether neurotransmitters or hormones are released through a transient (kiss & run) or an irreversibly dilating pore (full fusion) and provide a rationale as to how synaptic vesicles carrying 70 copies of v-SNAREs dock onto sites where as many t-SNAREs are clustered while only a few SNARE complexes are apparently enough to achieve fusion.

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Snare Mediated Fusion with Membrane Tension Control

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Fusion of membranes is ubiquitous in life. It is essential for neurotransmitter and hormone release, intracellular vesicular trafficking, fertilization, and viral infection. SNARE proteins constitute a highly conserved minimal fusion machinery mediating intracellular membrane merger from slow fusion of large yeast vacuoles (minutes) to extremely fast neurotransmitter release (<1 ms). While membrane tension was suggested to inhibit fusion by suppressing dimpling of membranes by viral fusion proteins (Markosyan et al., Biophys J, 1999), it was suggested to promote fusion pore opening and dilation in other studies (Shillcock and Lipowsky, Nat Mater, 2005; Nikolaus et al., Biophys J, 2010; Warner and O’shaughnessy, Biophys J, 2012). Thus, membrane tension may affect distinct stages of the fusion process differentially. To resolve how tension affects fusion, we established a fusion assay in which membrane tension is precisely controlled. Our approach is based on a previously established bulk assay in which v-SNARE reconstituted small liposomes (vSUVs) fuse to t-SNARE containing giant unilamellar vesicles (GUVs) (~10-30 micrometers in diameter) (Malsam et al., EMBO J, 2012). Using a micropipette, a single GUV is picked up, whose membrane tension is controlled by the aspiration pressure. Another pipette is maneuvered nearby to puff a suspension of vSUVs. Fusion is monitored as an increase of the GUV tongue projection in the aspiration pipette whose position can be determined with sub-pixel resolution. Because the