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# HIV fusion: Catch me if you can

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The penetration of enveloped viruses into target cells requires the fusion of the lipid envelope of their virions with the host lipid membrane through a stepwise and highly sophisticated process. However, the intermediate steps in this process have seldom been visualized due to their rarity and rapidity. Here, using cryo-electron tomography, TIRF microscopy, and cell membrane-derived vesicles called blebs, Ward *et al.* visualize intermediates of the HIV-cell membrane fusion process and demonstrate how Serinc proteins prevent full fusion by interfering with this process.

HIV is a major pathogen worldwide, with more than 38 million people living with HIV and 690,000 deaths reported in 2019 (<https://www.who.int/news-room/fact-sheets/detail/hiv-aids>, accessed July 6, 2020). HIV infection begins with entry of HIV particles into host cells through membrane fusion. This process is mediated by conformational changes of its virion surface glycoprotein, Env, induced upon binding to its receptor, CD4, and co-receptors, CCR5 and CXCR4 (1). The involvement of multiple biomolecules in this process, along with its rarity and rapidity, has made the study of this step highly challenging. The existence of other host molecules that can influence the entry process also complicates its investigation. For example, Serinc3 and Serinc5 are plasma membrane proteins involved in lipid synthesis and more specifically the incorporation of serine into both phosphatidylserine and sphingolipids. Regarding HIV fusion, it was shown that, in the absence of HIV Nef protein, Serinc proteins are incorporated into viral particles (2, 3), and some act as HIV entry inhibitors (2–4) that block membrane fusion. However, the exact mechanism by which they disrupt this process remains a matter of debate. Here, Ward *et al.* (5) describe a system to allow high-resolution observations of intermediate steps of fusion between HIV particles and the cellular plasma membrane, enabling new insights into the entry process and interrogations of membrane fusion blockers and specifically the Serinc3 and Serinc5 proteins. So far, the assumption that membrane fusion leads to opening of a pore enabling transit of the virus nucleocapsid was assessed by tracking fluorescent single particles in living cells by microscopy and, more specifically, by the loss of fluorescent HIV Gag protein signal due to its diffusion within the cells. It is noteworthy that cryo-electron tomography (cryoET) has previously been used with artificial membranes to visualize membrane fusion steps of influenza virus (6), which requires a much simpler acid-pH fusion trigger, opening the possibil-

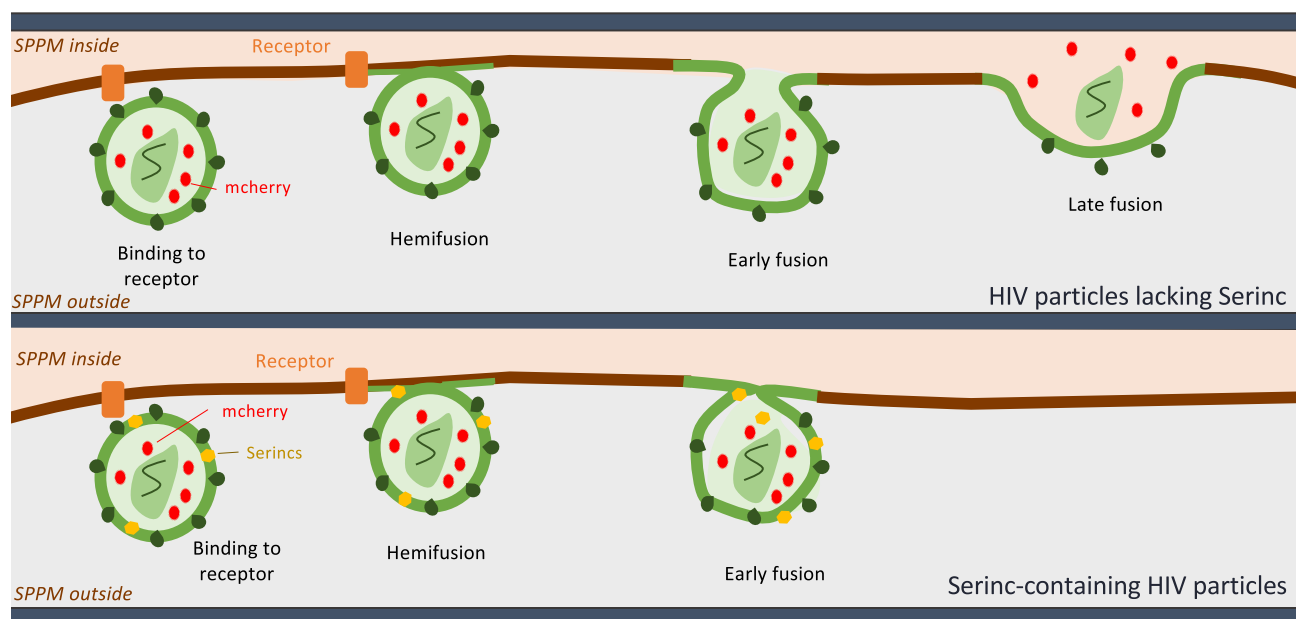
ity to study fusion of viruses that can directly fuse with the cell surface.

To delve more deeply into the membrane fusion process, Ward *et al.* began by developing a new methodological strategy combining total internal reflection fluorescence (TIRF) microscopy and cryoET. Because using whole cells is not adapted to the imaging of virus-plasma membrane fusion, the authors generated giant plasma membrane vesicles, or blebs, from donor cells expressing CD4 and CCR5 and CXCR4 that they modified to form so-called “supported planar plasma membranes” (SPPMs) (7). Specifically, they detected membrane fusion events by TIRF microscopy by using a soluble content marker protein incorporated within viral particles that, upon full membrane fusion, diffuse out of the virion structure. Then, using cryoET samples frozen after different delays corresponding to the timelines observed by TIRF microscopy, they captured membrane fusion events. Remarkably, they could detect intermediate steps of cell entry, including virion binding to receptors and hemifusion, as well as the final step of fusion, quantified via increases in the diameter of the bleb vesicles.

The authors next applied the two microscopy assays on blebs to investigate which steps of HIV membrane fusion were impacted by Serinc proteins. A comparison of viral particles with or without specific Serinc proteins revealed that fusion was impaired for Serinc3- and Serinc5-containing particles whereas Serinc2 had no impact on fusion. More particularly, they showed that Serinc3 and Serinc5 incorporation enhances the probability of hemifusion, which may lead to an abnormal early fusion resolution (*i.e.* without release of the viral nucleocapsid or soluble marker content), probably because the pore opening is too small or could not enlarge (Fig. 1). Experiments with the fluorescent lipid Atto488-DMPE showed that the dye diffuses into the bilayer, confirming that the process is stalled when or after hemifusion has occurred. The authors suspected that the defect was probably due to Serinc3- or Serinc5-mediated perturbation of lipid membrane. Indeed, the antifungal agent amphotericin B, known to induce changes in lipid membrane organization, could specifically restore membrane fusion of Serinc5- and likely Serinc3-containing viral particles.

Overall, Ward *et al.* showed for the first time the three-dimensional structures of intermediates of fusion between HIV virion and the plasma membrane. This is an important achievement for several reasons. First, this technological development is of utmost interest for the study of entry of a major pathogen and paves the way for the study of other viruses that require

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**Figure 1. A snapshot of HIV membrane fusion.** *Top*, particles lacking Serinc3/5 can fuse with the host cell, leading to cytoplasmic release of its nucleocapsid. *Bottom*, the presence of Serinc3 or Serinc5 on HIV particles prevents the completion of membrane fusion with the plasma membrane, inhibiting release.

activation by receptors or plasma membrane receptors. In addition, because the HIV packaging system is commonly used to generate pseudoparticles displaying a large variety of heterologous viral glycoproteins, this kind of tool will allow the study of different virus genera (8). Second, this study provides insights into the mechanisms by which Serinc3 and Serinc5 proteins can restrict HIV membrane fusion. Indeed, inhibition of infectivity by Serinc3 and Serinc5 was proposed to be linked to impairment of viral membrane distribution of Env or conformational changes required for fusion. Yet Ward *et al.* show that entry impairment is due to a reduced fusion pore, providing interesting clues as to the functions of Serinc's viral restriction. Beyond simply discovering viral restriction factors, this kind of characterization of host factors that block entry of infectious viral particles is key not only for unraveling the infection process but also for the development of antiviral drugs targeting host proteins. Finally, the study of the interplay between these cellular factors and HIV particles could highlight and provide insights into the physiological role of Serinc proteins. Indeed, the physiological function of the SERINC family remains poorly defined, and the difference between Serinc2 and Serinc3/5 on HIV entry suggests differential functions induced by its members. All in all, this study details new systems that can be used and extended in other fields of virology and cellular biology but also provides fantastic insights into HIV-host interactions.

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**Conflict of interest**—The authors declare that they have no conflicts of interest with the contents of this article.

**Abbreviations**—The abbreviations used are: cryoET, cryo-electron tomography; TIRF, total internal reflection fluorescence; SPPM, supported planar plasma membranes.

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