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HIV

Antiviral action countered by Nef

The HIV protein Nef is a viral 'Swiss army knife' with many functions. New work now shows how Nef increases infectivity — by inhibiting two of the host cell's antiviral proteins, SERINC3 and SERINC5.

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Nef is a small protein of the HIV virus that performs several diverse tasks during infection. It reduces the expression of a variety of proteins on the surface of the infected cell (usually, T cells of the host's immune system), modulates T-cell signalling pathways, and increases the infectivity of new virus particles released from the cell. But for more than two decades, the mechanism by which Nef achieves this last function has been poorly understood. Now, in two breakthrough studies reported on *Nature*'s website, Rosa *et al.*[1] and Usami *et al.*[2] show that Nef prevents the action of two host proteins previously not known to have antiviral activity: SERINC3 and SERINC5.

Work in the early 1990s showed that the ability of Nef to enhance viral infectivity is distinct from its well-characterized ability to downregulate the HIV receptor CD4, the removal of which is also important for infectivity. Although numerous studies had failed to find a strong effect of Nef on the structure or composition of HIV particles, one revealed that Nef had a mild enhancing effect on virus fusion with cells[3]. Other early studies showed that substituting the envelope proteins of HIV viruses, which mediate HIV entry into cells, with those of unrelated viruses can relieve the need for Nef in infection[4,5]. The effect of such envelope swapping — known as pseudotyping — was specific, because only some viral proteins relieved the requirement for Nef. An unusual study that used mixed viral particles also linked the requirement for Nef with the viral-envelope proteins[6]. Yet Nef's mechanism of enhancing infectivity remained elusive.

The Pizzato research group (who present the new paper by Rosa *et al.*) later uncovered two pieces of the puzzle, showing that Nef's action depends on dynamin, a host protein that is crucial for the internalization of cell-surface proteins[7], and that a glycosylated (carbohydrate-modified) variant of a structural protein from an unrelated retrovirus (the murine leukaemia virus (MLV) glyco-Gag protein) can also enhance HIV infectivity in a manner similar to that of Nef[8]. Although these clues did not reveal Nef's mechanism, they hinted that Nef had an effect on the cell, rather than on the virus particle. This may have pointed the investigators back to Nef's ability to modulate the levels of cell-surface proteins. The researchers behind the second of the two new papers (Usami *et al.*) subsequently showed[9] that HIV's dependence on Nef varies between strains of the virus and is linked to a specific domain in the HIV envelope protein gp120.

In the current studies, the two groups independently pursued different approaches that converged on the same answer: incorporation of the host proteins SERINC3 and SERINC5 into HIV particles reduces infectivity through a mechanism that is countered by Nef (Fig. 1). Rosa *et al.* quantified global gene expression in a panel of cell lines in which HIV exhibits either high or low dependence on Nef, and noted a strong correlation between Nef dependence and SERINC5 expression. By contrast, Usami and co-workers compared the protein composition of particles of normal HIV and Nef-defective HIV viruses that were released from cells in which virus infectivity strongly depends on Nef. This identified SERINC3 as a host protein that is enriched in particles that lack Nef.

Both SERINC3 and SERINC5 are members of a family of proteins named for a putative activity on membranes ('serine incorporator'). The SERINC proteins are integral membrane proteins that form scaffolds for enzymes involved in the synthesis of specific membrane phospholipid molecules[10]. The two research groups investigated all five family members, but found that SERINC3 and SERINC5 are the only ones to induce antiviral activity against HIV. Both Nef and MLV glyco-Gag were shown to counter the antiviral action of SERINC3 and SERINC5. The two SERINC

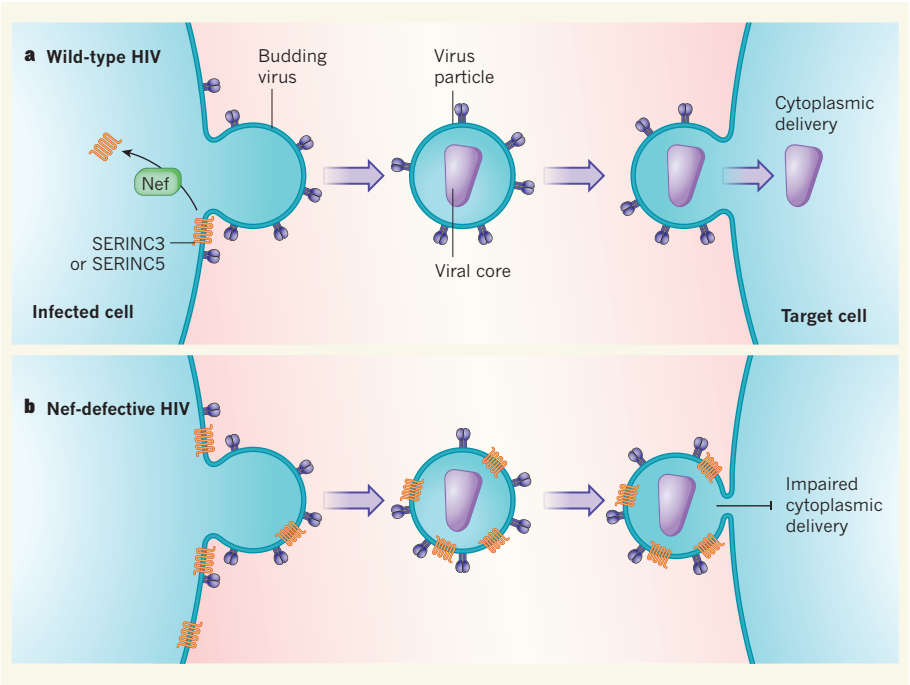

Figure 1 | SERINC proteins impair viral delivery. SERINC3 and SERINC5 are membrane proteins found by Rosa *et al.*[1] and Usami *et al.*[2] to have antiviral activity against HIV. **a**, The authors show that the HIV protein Nef prevents the SERINC proteins from being incorporated into a growing virus particle as it buds from the membrane of an infected cell. The resulting virus particle is able to correctly fuse with another target cell and deliver its viral core to the host-cell cytoplasm. **b**, The researchers propose that, in the absence of Nef, SERINC3 and SERINC5 are successfully incorporated into viral particles, and prevent delivery of the viral core by inhibiting the expansion of the fusion pore.

proteins are incorporated into Nef-defective HIV particles, but Nef and MLV glyco-Gag stop this from happening. They both induce SERINC5 to move from the cell surface to an intracellular compartment, preventing it from being incorporated into the budding virus, and demonstrating a plausible mechanism for how Nef enhances HIV infectivity.

The two groups also sought to determine whether pseudotyping HIV with Nef-independent surface proteins prevents the incorporation of SERINC3 or SERINC5 into Nef-defective HIV particles, but their different results leave this question open. However, Usami and colleagues demonstrated that the antiviral activity of SERINC3 and SERINC5 is specific for HIV surface proteins that cause virus infectivity to depend on Nef. Finally, depletion of SERINC3 or SERINC5 from host cells selectively enhanced the infectivity of Nef-defective HIV, confirming that SERINC proteins reduce HIV infectivity. Both SERINC3 and SERINC5 are expressed in human blood cells, suggesting that they are active in the primary targets of HIV infection *in vivo*.

How do SERINC3 and SERINC5 lower HIV infectivity? Both groups showed that, at high levels of expression, these proteins reduce the efficiency of virus fusion with target cells. However, the proteins inhibited HIV infectivity more strongly than they affected fusion, suggesting that they also affect an early post-fusion step in infection. Accordingly, Rosa *et al*. propose a model in which SERINC3 and SERINC5 prevent the expansion of the pore that is formed between the viral and cell membranes and that is necessary for delivery of the viral core into the cell's cytoplasm (Fig. 1). However, it is not clear whether this would result in the impaired reverse transcription of the viral genome that is normally seen in Nef-defective HIV. One intriguing possibility is that the impeded viral core is targeted for cellular destruction, as previously suggested[11].

The identification of SERINC3 and SERINC5 as antiviral proteins that are counteracted by diverse retroviruses suggests that these proteins may also target other enveloped viruses, which may in turn have different mechanisms for escaping their antiviral action. These two proteins can therefore be used as probes to examine the entry mechanisms of enveloped viruses. Although the available data suggest that these proteins target specific regions of viral glycoproteins, it is possible that they inhibit fusion indirectly by controlling the lipid composition or fluidity of the viral membrane. Regardless of the specific antiviral mechanism, the ability of Nef to counteract SERINC3 and SERINC5 adds to the impressive list of functions of this remarkable little viral protein. ■

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