

## RESTRICTION OF HIV-1 VIRION-RELEASE BY BST-2 AND ITS COUNTERACTION BY VPU

John Guatelli, Kathleen Fitzpatrick, David Lau, Jason Munguia, Mark Skasko, Andrey Tokarev, John Crum, Thomas Deerinck, and Mark Ellisman

Departments of Medicine and Neurosciences, University of California San Diego, and the San Diego Veterans Affairs Healthcare System, La Jolla, CA 92093-0679, USA

The innate response to viral infection includes the induction of host factors that restrict viral replication but are counteracted by specific viral gene products. Investigation of the Vpu protein of HIV-1 recently led to the discovery of a novel mechanism of such restriction: retention of progeny virions on the surface of infected cells by the interferon-induced, transmembrane and GPI-anchored protein BST-2 (CD317; tetherin). BST-2 is a broad-spectrum inhibitor of enveloped viruses that targets members of the retrovirus, arenavirus, and filovirus families, but how it restricts viral release and is counteracted by viral gene products is unclear.

Here, electron microscopic data suggest that BST-2 directly retains nascent HIV-1 virions at the plasma membrane and is incorporated into virions. Virion-incorporation was confirmed by capture of infectious virus from solution using antibody specific to the ectodomain of BST-2. Immunoblot analysis of partially purified virions supported the incorporation of BST-2 and suggested that Vpu reduces but does not eliminate this.

The relief of restricted virion release by Vpu is associated with the down-regulation of BST-2 from the cell surface. This effect is mediated in part through an interaction between Vpu and the cellular protein  $\beta$ -TrCP, a substrate adaptor for a specific E3 ubiquitin ligase complex. Consistent with this, Vpu stimulated the ubiquitination of BST-2. The optimal down-regulation of BST-2 by Vpu required the clathrin adaptor AP-2 and endosomal acidification, suggesting that Vpu removes BST-2 from its site of action at the plasma membrane via ubiquitin-mediated endo-lysosomal trafficking.

A direct model of restriction can potentially be generalized to all enveloped viruses that assemble on plasma membrane domains that contain BST-2. Conversely, a model of relief of restriction by removal of BST-2 from these sites and from virions can potentially be generalized to all viral proteins that decrease the expression of BST-2 at the plasma membrane. The inhibition of BST-2 antagonists such as Vpu may represent new approaches to the treatment of infections due to enveloped viruses.