VIROLOGY

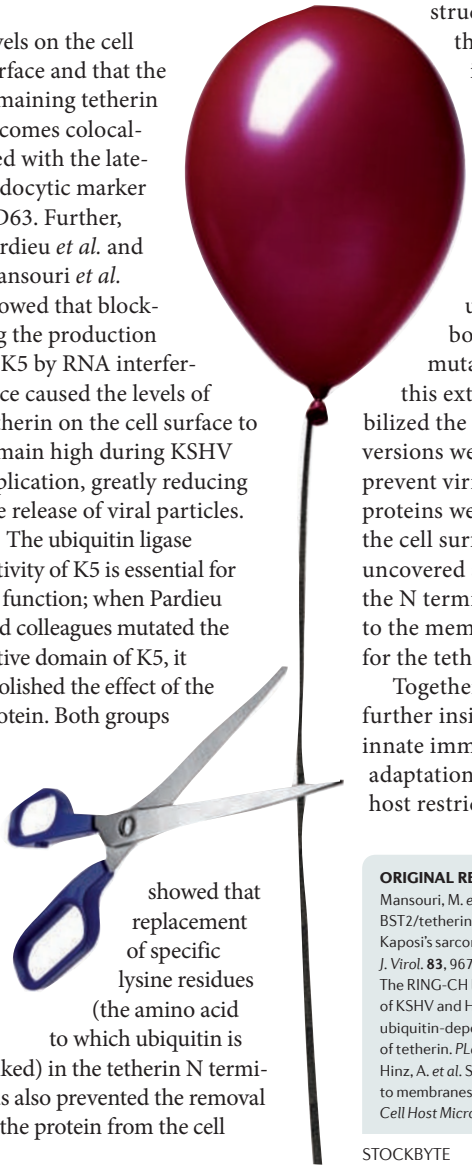
Cleaving the tether

> K5 protein from Kaposi's sarcoma-associated herpesvirus … promotes viral release by reducing the levels of tetherin through ubiquitylation

Tetherin (also known as BST2) restricts enveloped viruses by preventing their release from the surface of infected cells. However, several viruses have developed strategies to circumvent this restriction. Mansouri *et al.* and Pardieu *et al.* now show that the K5 protein from Kaposi's sarcoma-associated herpesvirus (KSHV; also known as human herpesvirus 8 (HHV8)) promotes viral release by reducing the levels of tetherin through ubiquitylation, and Hinz and colleagues provide further insight into the mechanism of action of tetherin.

Tetherin is a dimeric type II transmembrane protein with a short amino-terminal cytoplasmic tail and an extracellular domain that is anchored to the membrane at the carboxyl terminus by a glycosyl phosphatidylinositol anchor. When a budding viral particle incorporates a membrane anchor, the virion remains tethered to the membrane and can be subsequently endocytosed. Previous experiments had determined that the levels of tetherin and other immune-related proteins were decreased after expression of KSHV K5, a membrane-associated RING-CH (MARCH) E3 ubiquitin ligase, hinting at a possible role for tetherin and K5 in viral replication. Pardieu and colleagues now show that expression of K5 lowers tetherin levels on the cell surface and that the remaining tetherin becomes colocalized with the late-endocytic marker CD63. Further, Pardieu *et al.* and Mansouri *et al.* showed that blocking the production of K5 by RNA interference caused the levels of tetherin on the cell surface to remain high during KSHV replication, greatly reducing the release of viral particles.

The ubiquitin ligase activity of K5 is essential for its function; when Pardieu and colleagues mutated the active domain of K5, it abolished the effect of the protein. Both groups showed that replacement of specific lysine residues (the amino acid to which ubiquitin is linked) in the tetherin N terminus also prevented the removal of the protein from the cell surface and that tetherin degradation requires proteasome activity.



To determine how tetherin restricts viruses, Hinz and colleagues investigated the extracellular domain biochemically and structurally. They found that this domain dimerizes, even when its cysteine crossbridges are removed. The crystal structure of the domain showed that the protein forms a coiled coil, as predicted, with an unusual disulphide bond-dependent stability; mutagenesis of residues in this extracellular domain destabilized the coiled coil. The mutated versions were also unable to prevent virion release, although the proteins were correctly localized to the cell surface. Mutagenesis also uncovered another region, closer to the N terminus and thus closer to the membrane, that is required for the tethering function.

Together, these studies provide further insight into an important innate immune molecule and the adaptation of a virus to circumvent host restriction.

Christiaan van Ooij

ORIGINAL RESEARCH PAPERS
Mansouri, M. *et al.* Molecular mechanism of BST2/tetherin downregulation by K5/MIR2 of Kaposi's sarcoma-associated herpesvirus. *J. Virol.* **83**, 9672–9681 (2009) | Pardieu, C. *et al.* The RING-CH ligase K5 antagonizes restriction of KSHV and HIV-1 particle release by mediating ubiquitin-dependent endosomal degradation of tetherin. *PLoS Pathog.* **6**, e1000843 (2010) | Hinz, A. *et al.* Structural basis of HIV-1 tethering to membranes by the BST-2/tetherin ectodomain. *Cell Host Microbe* **7**, 314–323 (2010)

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