plasmid construction, protein expression, crystallization, and structure determination are provided in the Supplemental Data.

Protein-Protein Interaction Experiments

Biosensor binding experiments were performed as described (Garrus et al., 2001) with purified HIV-1 $p6^{Gag}$, EIAV $p9^{Gag}$, and HIV-1 $TSG101_{UEV}$ proteins binding to immobilized GST-$ALIX_{Bro1\text{-}V}$, GST-$ALIX_{V}$, GST-$ALIX_{Bro1}$, and GST-$ALIX_{714–723}$ proteins. Assay conditions and binding affinities are provided in Table S2. GST pulldown experiments were performed as described (von Schwedler et al., 2003) using purified $ALIX_{Bro1}$ proteins binding to GST or GST-CHMP4A proteins captured from clarified *E. coli* lysates.

Assays for HIV-1 ΔPTAP Release and Infectivity

293T cells (~8×10^5 cells/well in 6-well plates) were transfected with 1 μg of HIV-1 ΔPTAP plasmid (Garrus et al., 2001) + 1 μg of ALIX expression vector per well (10 μl Lipofectamine 2000, Invitrogen). Cytoplasmic proteins and sucrose-pelleted virions were harvested 24 hr posttransfection, analyzed by western blotting, and quantified using an Odyssey imaging system (Li-COR, Inc.). Primary antibodies were rabbit anti-CA and rabbit anti-MA at 1:15,000. HIV-infectious titers were assayed in single-cycle MAGIC assays in P4 cells. Additional experimental details are provided in von Schwedler et al. (2003).

EIAV VLP Production and ALIX Silencing

HeLa M cells (~4×10^5 cells/well, 6-well plate) were transiently transfected (10 μl; FuGene6, Roche Applied Science) with 5 μg of wild-type or $p9^{Gag}$ ΔYP pEV53B EIAV vector (Olsen, 1998). EIAV virus-like particles were harvested 48 hr posttransfection, concentrated through 20% sucrose cushions, and analyzed by western blotting using affinity-purified EIAV anti-CA antibody (1:10,000). For ALIX-depletion experiments, an shRNA targeting human ALIX nucleotides 1765–1783 (Chen et al., 2005) was delivered using the FG12 lentiviral expression vector (Qin et al., 2003; 8 μg/ml polybrene, moi = 15) 24 hr prior to transfection with pEV53B.

Supplemental Data

Supplemental Data include Experimental Procedures, References, five figures, and two tables and can be found with this article online at http://www.cell.com/cgi/content/full/128/5/841/DC1/.

ACKNOWLEDGMENTS

We thank Michael Landesman for performing the yeast two-hybrid experiment shown in Figure S5 and Heidi Schubert, Andrew VanDemark, and Frank Whitby for helpful guidance in the crystallography. Operations of the National Synchrotron Light Source (NSLS) are supported by the Office of Basic Energy Sciences at the U.S. Department of Energy and by the National Institutes of Health (NIH). Data collection at the NSLS was funded by the National Center for Research Resources. This work was supported by NIH Grants GM066521 (C.P.H. and W.I.S.) and AI051174 (W.I.S.).

Received: November 13, 2006
Revised: December 22, 2006
Accepted: January 5, 2007
Published: March 8, 2007

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