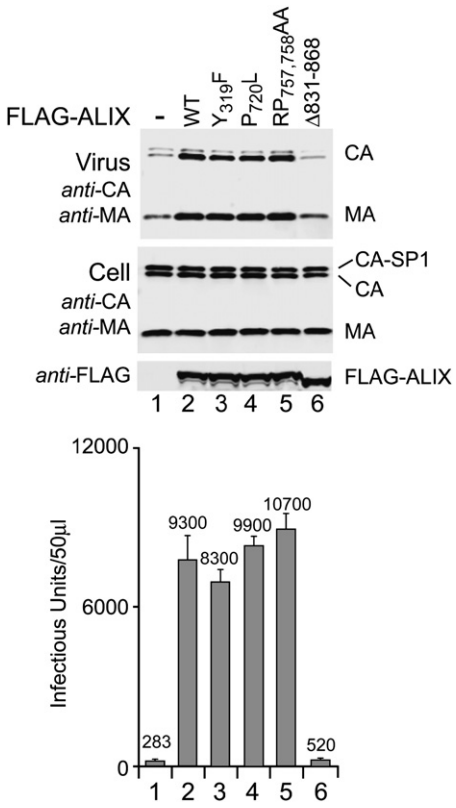

Figure 7. ALIX Requirements for HIV-1 ΔPTAP Release and Infectivity

HIV-1 ΔPTAP release and infectivity are shown when coexpressed with: an empty vector (−; lanes 1) with expression vectors for wild-type ALIX (lanes 2), with the ALIX $Y_{319}F$ mutant (lanes 3), which cannot be phosphorylated on residue 319, with the $ALIX_{P720L}$ mutant (lanes 4), which is defective in TSG101 binding, with the $ALIX_{RP757,758AA}$ mutant (lanes 5), which is defective in endophilin binding, or with the $ALIX_{\Delta 831-868}$ mutant (lanes 6), which lacks the final 38 residues in the PRR. HIV-1 ΔPTAP release, cellular Gag protein levels, exogenous ALIX expression, and viral infectivity were analyzed as described in Figure 4, with error bars representing standard deviations from three separate infectivity experiments.

depleted from cells (not shown), which indicates that the ALIX and TSG101 virus-budding pathways can function independently.

Endophilins represent another interesting class of ALIX-binding partners because endophilins can bind membranes and can drive (or sense) membrane curvature. Moreover, endophilin-2 binds and facilitates MLV Gag protein release (Wang et al., 2003). Endophilins bind the $_{755}PXRPPPP_{761}$ sequence within the ALIX PRR (Chatellard-Causse et al., 2002; Shibata et al., 2004). We employed yeast two-hybrid assays to confirm that the ALIX $RP_{757,758}AA$ mutation inhibited binding to both endophilin-1 and -2 (Figure S5B). Despite lacking endophilin-binding activity, however, the $ALIX_{RP757,758AA}$ mutant efficiently rescued HIV-1 ΔPTAP release and infectivity, which indicates that endophilin binding was also dispensable for the virus-budding function of ALIX (Figure 7, lane 5).

Finally, we tested the general importance of the ALIX PRR using several deletion mutants, the shortest of which removed just the final 38 ALIX residues ($ALIX_{\Delta 831-868}$). All of the C-terminal deletion mutants tested, including $ALIX_{\Delta 831-868}$, failed to rescue HIV ΔPTAP release and infectivity (lane 6) despite equivalent expression levels of the mutant and wild-type proteins (panel 3, compare lanes 2 and 6). We therefore conclude that ALIX PRR interaction(s) are required for late budding activity.

DISCUSSION

$ALIX_{Bro1-V}$ Structure

The elongated Bro1 domain extends from the first arm of the central V domain, which gives $ALIX_{Bro1-V}$ the shape of a "check mark." This distinctive conformation explains how Src kinase can bridge the N-terminal Bro1 domain and the distal PRR tail and how it may similarly bring other Bro1- and PRR-binding proteins into close proximity. The $ALIX_{Bro1-V}$ structure also suggests several ways in which conformational changes could function in the numerous biological roles ascribed to ALIX. Two types of domain motion are attractive possibilities: (1) variation in the relative orientation of the Bro1 and V domains, which appears possible given the limited number of contacts made by the interdomain linker (Figure 2A), and (2) variation in the trajectories of the two arms of the V domain, as implied by differences in the three different V-domain structures (Figure 2B).

In addition to the modest variation in arm orientation observed between crystal structures, we speculate that the V domain might function as a "molecular hinge," with the structures reported here corresponding to the closed conformation. This idea is consistent with the lack of hydrophobic packing interactions throughout the loop region of the V domain (Figure 2C). The hydrophilic loop-packing interactions that do exist are well defined and geometrically constrained, however, which indicates that if the loop functions as a hinge, then activation energy will be required to break these interactions and open the V. In principle, hinge opening could be driven by posttranslational modifications and/or ligand binding and might expose new binding sites and thereby provide an elegant mechanism for transmitting signals for complex assembly and disassembly. YPX_nL late domains are obvious candidates for triggering a possible hinge-opening motion because they bind near the base of the V at a site located nearly between the two arms.

YPX_nL Binding

We have mapped the YPX_nL-binding site to a conserved, hydrophobic groove on arm2 of the ALIX V domain and demonstrated that YPX_nL binding is required for ALIX-mediated budding of both HIV-1 and EIAV. ALIX binds nearly 60× more tightly to EIAV $p9^{Gag}$ than to HIV-1 $p6^{Gag}$, which is consistent with the idea that the EIAV $_{23}YPDL_{26}$ late