lysobisphosphatidic acid (LBPA; reviewed in van der Goot and Gruenberg, 2006); (2) endocytosis via interactions with the membrane-curvature-sensing endophilins (Chatellard-Causse et al., 2002; Gallop and McMahon, 2005); (3) cell-surface-receptor downregulation via direct interactions with cell-surface receptors (Geminard et al., 2004) and through antagonism of the Cbl-SETA/CIN85-endophilin complex (Schmidt et al., 2004); (4) spatial distribution of endosomes via regulation of cortical actin (Cabezas et al., 2005); (5) cell motility/adhesion via interactions with FAK (*f*ocal *a*dhesion *k*inase), PYK2 (*p*roline-rich *t*yrosine *k*inase *2*; Schmidt et al., 2003), and possibly also RabGAPLP (*Rab G*TPase-*a*ctivating *p*rotein-*l*ike *p*rotein; Ichioka et al., 2005); (6) apoptosis via interactions with the calcium-binding EF-hand protein ALG-2 (*a*poptosis-*l*inked *g*ene-2; reviewed in Sadoul, 2006); (7) regulation of the JNK signaling pathway via interactions with ALG-2 and the ubiquitin E3 ligase POSH (*p*lenty *o*f *SH*3 domains; Tsuda et al., 2006). The latter observation is of particular interest because POSH is also required for HIV-1 release (Alroy et al., 2005), which raises the possibility that the ALIX-POSH-ALG-2 complex could function in HIV-1 budding.

In summary, it is now apparent that ALIX performs a series of important functions in the endosomal pathway, in cytoskeletal dynamics, and in enveloped virus budding. However, the mechanistic bases for these seemingly diverse ALIX functions are not known. Our studies were therefore undertaken with the goals of elucidating the structure of ALIX, determining how it interacts with retroviral Gag proteins, and testing its requirements for functioning in virus budding.

RESULTS

Structural Studies of Human ALIX

As illustrated in Figure 1A, human ALIX can be subdivided into three regions; the Bro1 domain (residues 1–358), the "V" domain (362–702), and the proline-rich region (PRR; 703–868). Constructs containing the PRR did not express well in *E. coli*, but constructs spanning the Bro1 and V domains could be expressed and purified. A series of $ALIX_{Bro1-V}$ proteins with loop mutations were surveyed to identify a protein that would crystallize. The $KK_{268,269}YY$ mutation enabled crystallization (apparently because Tyr268 makes an important crystal contact), and the structure of this protein (hereafter termed $ALIX_{Bro1-V}$) was determined at a resolution of 3.3 Å (R_{free} = 31.7%; Figures 1B and S1; Table S1). Isolated wild-type Bro1 and V domains were also crystallized and their structures were determined at higher resolutions (2.55 Å, R_{free} = 27.4% and 2.6 Å, R_{free} = 30.2%, respectively; see Figures 1C–1E). The V-domain crystals contained two molecules in the asymmetric unit, and the different structures therefore provided three independent views of the ALIX V domain.

Global Architecture of $ALIX_{Bro1-V}$

As illustrated in Figure 1B, the $ALIX_{Bro1-V}$ protein adopts an extended conformation in which the Bro1 and V domains form discrete elements. Like its yeast analog, Bro1p (Kim et al., 2005), $ALIX_{Bro1}$ is shaped like a banana, with a long dimension of ~100 Å. The domain is organized about a core tetratricopeptide repeat (TPR) composed of three helical hairpins that associate into a right-handed superhelix (Figure 1C). The ALIX V domain is composed of two extended arms that fold back on themselves at an angle of ~30° to form a V. The arms are 77 Å (arm1) and 90 Å (arm2) in length, and each is organized about an extended three-helix bundle (Figures 1D and 1E). The V-shaped conformation brings the N-terminal Bro1 domain and the C-terminal PRR (not present in the structure) into spatial proximity, thereby explaining how Src kinase can simultaneously dock to the Bro1 domain and phosphorylate Tyr residues in the ALIX C-terminal tail (Schmidt et al., 2005).

$ALIX_{Bro1-V}$ has a highly asymmetric shape because the long axes of the Bro1 and arm1 elements connect in parallel. As a result, $ALIX_{Bro1-V}$ is ~150 Å in its longest dimension but less than 50 Å in its other two. Small-angle X-ray scattering profiles fit the crystal model well (R.D.F. and H. Tsuruta, unpublished data), which indicates that the crystal structure provides a good model for the solution structure of the uncomplexed protein. Nevertheless, we speculate that the different domains could change their relative orientations in response to environmental cues like ligand binding because the Bro1 and V domains are connected by a single tripeptide linker ($_{359}VPV_{361}$) that makes only limited noncovalent contacts (Figures 1B and 2A). Moreover, the V-domain arm trajectories vary in the three different structures, which results in relative displacements of up to 10 Å at their tips (Figure 2B). Finally, the loop region between the two arms of the V domain also has the potential to act as a hinge (Figure 2C and Discussion). Thus, the overall impression is that $ALIX_{Bro1-V}$ is a scaffold composed of extended domains that may reorient in response to ligand binding.

Structure of the Bro1 Domain

The three helical hairpins that comprise the $ALIX_{Bro1}$ TPR core (α4/5, α6/7, and α8/9) are each ~50 residues in length. They form a right-handed solenoid with a rotation of ~20° between each helical pair. The core is flanked on one side by α10 (left in Figure 1C) and on the other by a small β sheet (β1, β2) and a three-helix bundle (α1–3). Both the N and C termini adopt extended conformations that traverse opposite sides of the domain, with the first 17 residues extending along the convex surface and the final 43 residues extending across the concave surface.

As expected, human $ALIX_{Bro1}$ generally resembles its yeast counterpart (Kim et al., 2005), with an overall rmsd of 2.8 Å over 338 equivalent Cα positions (Figure S2). The character of the domain surface is also largely conserved, with two exposed hydrophobic patches centered about Tyr319 and Phe199. The first patch forms the docking site for Src kinase when Tyr319 is phosphorylated (Schmidt et al., 2005), and the second patch forms the binding site for the CHMP4B subunits of the ESCRT-III complex (Kim et al., 2005 and see below). A strongly