Cell

Structural and Biochemical Studies of ALIX/AIP1 and Its Role in Retrovirus Budding

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DOI 10.1016/j.cell.2007.01.035

SUMMARY

ALIX/AIP1 functions in enveloped virus budding, endosomal protein sorting, and many other cellular processes. Retroviruses, including HIV-1, SIV, and EIAV, bind and recruit ALIX through YPX_nL late-domain motifs (X = any residue; n = 1–3). Crystal structures reveal that human ALIX is composed of an N-terminal Bro1 domain and a central domain that is composed of two extended three-helix bundles that form elongated arms that fold back into a "V." The structures also reveal conformational flexibility in the arms that suggests that the V domain may act as a flexible hinge in response to ligand binding. YPX_nL late domains bind in a conserved hydrophobic pocket on the second arm near the apex of the V, whereas CHMP4/ESCRT-III proteins bind a conserved hydrophobic patch on the Bro1 domain, and both interactions are required for virus budding. ALIX therefore serves as a flexible, extended scaffold that connects retroviral Gag proteins to ESCRT-III and other cellular-budding machinery.

INTRODUCTION

Many enveloped RNA viruses use short peptide motifs, termed "late domains," to recruit cellular factors that facilitate budding (reviewed in Bieniasz, 2006; Demirov and Freed, 2004; Morita and Sundquist, 2004). Two of the best characterized late domains are the PTAP motif, which binds and recruits TSG101 (*t*umor *s*usceptibility *g*ene 101; Demirov et al., 2002; Garrus et al., 2001; Gottlinger et al., 1991; Huang et al., 1995; Martin-Serrano et al., 2001; VerPlank et al., 2001), and the YPX_nL motif (where X can vary in sequence and length), which binds ALIX/AIP1 (*AL*G-2-*i*nteracting protein *X*; Chen et al., 2005; Puffer et al., 1997; Strack et al., 2003; Vincent et al., 2003). YPX_nL late domains can vary in sequence and can function alone or together with PTAP late domains. For example, the structural $p6^{Gag}$ protein of HIV-1_{NL4-3} contains $_{7}PTAP_{10}$ and $_{36}YPLASL_{41}$ late domains that function in tandem, whereas the analogous EIAV $p9^{Gag}$ protein contains a single $_{23}YPDL_{26}$ late domain. In principle, multiple late domains could synergistically enhance virus release and/or expand viral tropism.

Both TSG101 (Vps23p in yeast) and ALIX (Bro1p) help sort membrane proteins into vesicles that bud into the lumen of multivesicular bodies (MVB), which supports the idea that virus budding and MVB vesicle formation are highly related processes. The requirements for MVB protein sorting and vesicle formation are best understood in yeast, where the process requires the action of at least 18 different "Class E" proteins. Most, but not all, Class E proteins are stable subunits of the three ESCRT complexes (*e*ndosomal *s*orting *c*omplexes *r*equired for *t*ransport; Hurley and Emr, 2006). Humans have at least one homolog of every yeast Class E protein, and MVB vesicle sorting therefore appears to be a highly conserved process, albeit one that occurs with considerably greater complexity in mammals.

TSG101 functions as the central subunit of ESCRT-I, which recognizes ubiquitylated membrane-protein cargoes and helps recruit the downstream machinery necessary for protein sorting and MVB vesicle formation (Hurley and Emr, 2006). This downstream machinery includes the ESCRT-III and VPS4-LIP5 complexes, which appear to be intimately involved in the actual mechanics of protein sorting and vesicle formation. ALIX is also a Class E protein and can interact directly with both ESCRT-I and ESCRT-III but is not a stable subunit of either complex (reviewed in Odorizzi, 2006). The yeast homolog Bro1p also recruits the deubiquitylating enzyme Doa4p, which removes ubiquitin from cargoes as they are sorted into MVB vesicles (Dejournett et al., 2006; Luhtala and Odorizzi, 2004).

In addition to its roles in virus budding and MVB cargo sorting, ALIX has also been implicated in a number of other important cellular processes, including (1) MVB vesicle fission and back fusion via regulation of the conical lipid,