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The Vps27/Hse1 Complex Is a GAT Domain-Based Scaffold for Ubiquitin-Dependent Sorting

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SUMMARY

The yeast Vps27/Hse1 complex and the homologous mammalian Hrs/STAM complex deliver ubiquitinated transmembrane proteins to the ESCRT endosomal-sorting pathway. The Vps27/Hse1 complex directly binds to ubiquitinated transmembrane proteins and recruits both ubiquitin ligases and deubiquitinating enzymes. We have solved the crystal structure of the core responsible for the assembly of the Vps27/Hse1 complex at 3.0 Å resolution. The structure consists of two intertwined GAT domains, each consisting of two helices from one subunit and one from the other. The two GAT domains are connected by an antiparallel coiled coil, forming a 90 Å-long barbell-like structure. This structure places the domains of Vps27 and Hse1 that recruit ubiquitinated cargo and deubiquitinating enzymes close to each other. Coarse-grained Monte Carlo simulations of the Vps27/Hse1 complex on a membrane show how the complex binds cooperatively to lipids and ubiquitinated membrane proteins and acts as a scaffold for ubiquitination reactions.

INTRODUCTION

Protein ubiquitination is a widespread, multifunctional regulatory mechanism. Ubiquitin is conjugated to proteins via an isopeptide bond between the C terminus of ubiquitin and Lys residues in the ubiquitinated protein. This reaction is carried out by a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin protein ligase (E3) (Hershko et al., 2000; Hochstrasser, 2000; Pickart, 2001; Weissman, 2001). Ubiquitination is a major regulator of endocytosis and vesicular trafficking (Hicke, 2001; Raiborg et al., 2003). Ubiquitinated proteins are targeted to and regulate the vesicular trafficking machinery via interactions between the ubiquitin moiety and proteins that contain ubiquitin-binding domains (Harper and Schulman, 2006; Hicke et al., 2005; Hurley et al., 2006).

The ESCRT protein network targets ubiquitinated transmembrane proteins for degradation in the lysosome or yeast vacuole (Babst, 2005; Bowers and Stevens, 2005; Hurley and Emr, 2006; Slagsvold et al., 2006). These proteins were discovered in yeast, in which defects in their genes lead to an enlarged cargo-rich compartment adjacent to the vacuole (Bowers and Stevens, 2005). This phenotype is referred to as a class E vacuolar protein-sorting (VPS) defect. Yeast class E *VPS* genes encode the subunits of four hetero-oligomeric protein complexes: the Vps27/Hse1 complex (Bilodeau et al., 2003; Bowers and Stevens, 2005; Piper et al., 1995) and ESCRT-I, -II, and -III (Babst, 2005; Bowers and Stevens, 2005; Hurley and Emr, 2006; Slagsvold et al., 2006). The ESCRT network is conserved from yeast to human and sorts ubiquitinated transmembrane proteins into small vesicles that bud into the lumen of endosomes, thus forming multivesicular bodies (MVBs) (Gruenberg and Stenmark, 2004; Piper and Luzio, 2001). In mammalian cells, the ESCRT network directs the lysosomal degradation of signaling molecules such as the EGF receptor (Clague and Urbe, 2001; Haglund et al., 2003; Katzmann et al., 2002; Slagsvold et al., 2006). Further, this network is hijacked by viruses such as HIV, which use a process topologically equivalent to MVB formation to bud from cells (Demirov and Freed, 2004; Morita and Sundquist, 2004).

Vps27/Hse1 is a multifunctional complex required for MVB sorting of ubiquitinated cargo molecules as well as the efficient recycling of late Golgi proteins including the carboxypeptidase Y (CPY) sorting receptor, Vps10 (Bilodeau et al., 2002, 2003; Piper et al., 1995). Human Vps27 is known as Hrs (hepatocyte growth factor receptor substrate), and Hse1 has two human orthologs, STAM1 and STAM2 (signal-transducing adaptor molecule) (Komada and Kitamura, 2005) (Figure 1A). The Vps27/Hse1 and Hrs/STAM complexes sort cargo proteins from early endosomes to the ESCRT-I complex (Bilodeau et al., 2003; Katzmann et al., 2003) via clathrin-coated domains (Lloyd et al., 2002; Raiborg et al., 2002). The Vps27/Hse1 complex is targeted to early endosomes via the FYVE domains of Vps27 or Hrs (Raiborg et al., 2001), which bind to phosphatidylinositol 3-phosphate (PI(3)P). The Vps27/Hse1

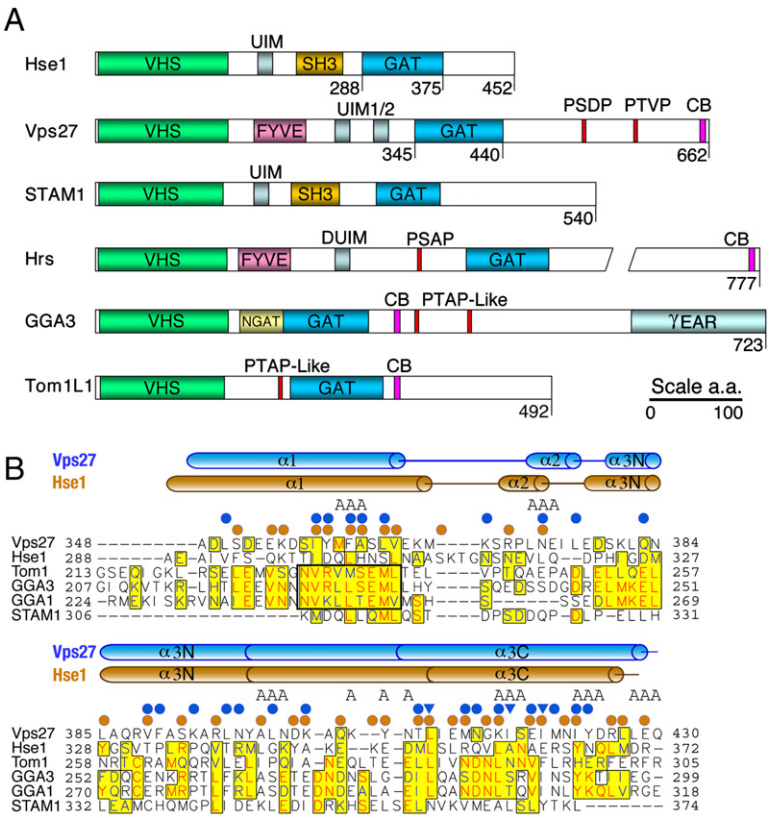

Figure 1. Modular Organization of Vps27, Hse1, and Related Proteins and Alignment of GAT Domains

(A) Modular organization of Vps27, Hse1, and other GAT-domain-containing proteins. Domain name abbreviations are as follows: VHS, Vps27/Hrs/STAM; UIM, ubiquitin-interacting motif; SH3, Src homology-3; GAT, GGA and TOM; GGA, Golgi-localized, γ-ear-containing, ADP-ribosylation-factor-binding protein; TOM, target of Myb; FYVE, Fab1/YOTP/Vac1/EEA1; CB, clathrin-binding; DUIM, double UIM; NGAT, the N-terminal region preceding the GAT domain, responsible for binding to Arf1-GTP; GAE, γ-adaptin ear. A helical region of Hrs is a putative, but unproven, GAT domain.

(B) GAT domains were aligned based on three-dimensional structural superposition where available (Vps27, Hse1, GGA1, GGA3, and Tom1), and otherwise by sequence homology with the most similar protein of known structure. Colored dots or triangles indicate residues of Vps27 (blue) and Hse1 (orange) that participate in the heterodimer interface. Residues shown in triangles were mutated in this study. The letter "A" above the alignment denotes residues of Vps27 mutated to Ala (Bilodeau et al., 2003). The major site 1 ubiquitin-binding motif of GGA1, GGA3, and Tom1 as discussed in the text is outlined in black.

complex recruits clathrin via a short peptide motif near the C termini of Vps27 and Hrs (Raiborg et al., 2002), and both proteins contain P(S/T)XP motifs that recruit ESCRT-I (Bilodeau et al., 2003; Katzmann et al., 2003; Lu et al., 2003).

The Vps27/Hse1 and Hrs/STAM complexes are scaffolds for binding of ubiquitinated cargo proteins and coordinating ubiquitination and deubiquitination reactions that regulate sorting. The yeast complex recruits ubiquitinated cargo via two tandem ubiquitin-interacting motifs (UIMs) in Vps27 (Bilodeau et al., 2002; Shih et al., 2002; Swanson et al., 2003) and one in Hse1. Hse1 and STAM isoforms recruit the deubiquitinating enzymes (DUBs) UBPY (Kaneko et al., 2003; Kato et al., 2000), AMSH (McCullough et al., 2006), and, in yeast, Ubp7 (Ren et al., 2007) through their SH3 domains. The Hse1 SH3 domain also recruits the adaptor protein Hua1, which, in turn, recruits a complex of the ubiquitin ligase Rsp5, the DUB Ubp2, and the regulatory protein Rup1 (Ren et al., 2007). There is a second mechanism in which the C terminus of Hse1 binds the ubiquitin ligase Rsp5 directly (Bowers et al., 2004; Ren et al., 2007). These mechanisms regulate different cargo to different extents. CPY sorting is slowed, but not blocked, when the complex is disrupted by the loss of Hse1 (Bilodeau et al., 2002). In contrast, cargo such as carboxypeptidase S (Cps1) and the mating factor receptor Ste3 are profoundly dependent on the integrity of the complex, and their sorting is largely blocked by loss of Hse1 (Bilodeau et al., 2002; Ren et al., 2007).

While much is known about individual domains of the Vps27/Hse1 complex, little is known about how the subunits of the complex associate with one another. A predicted coiled-coil region in the Vps27/Hse1 (Bilodeau et al., 2003) and Hrs/STAM (Mizuno et al., 2004) complexes is necessary for complex formation, and an adjacent region of STAM1 dubbed the "STAM-specific motif" (SSM) has also been implicated (Mizuno et al., 2004). To characterize the core around which the complex assembles, we carried out sequence-similarity searches in the region of the predicted coiled coil by using PSI-Blast (Altschul et al., 1997). To our surprise, these regions of Vps27, Hse1, and STAM all showed statistically significant similarity (E values of $\sim 10^{-9}$) to the GAT (GGAs and TOM) domains of the GGA and TOM trafficking adaptor proteins (Figures 1A and 1B). GAT domains are monomeric three-helix bundles (Collins et al., 2003; Shiba et al., 2003; Suer et al., 2003; Zhu et al., 2003) that bind to ubiquitin (Mattera et al., 2004; Puertollano and Bonifacino, 2004; Scott et al., 2004; Shiba et al., 2004). We sought to test the prediction that Vps27 and Hse1 contain GAT domains and to understand how a GAT domain-based core could assemble an oligomeric complex by determining the crystal structure of what we refer to as the Vps27/Hse1 core complex. The structure shows that the core complex consists of two intertwined GAT domains, each consisting of two helices from one subunit and one from the other. The two GAT domains are connected by a two-stranded coiled coil. Residues in the interface between the subunits