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The vacuolar protein sorting genes in insects: A comparative genome view

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ABSTRACT

In eukaryotic cells, regulated vesicular trafficking is critical for directing protein transport and for recycling and degradation of membrane lipids and proteins. Through carefully regulated transport vesicles, the endomembrane system performs a large and important array of dynamic cellular functions while maintaining the integrity of the cellular membrane system. Genetic studies in yeast *Saccharomyces cerevisiae* have identified approximately 50 vacuolar protein sorting (VPS) genes involved in vesicle trafficking, and most of these genes are also characterized in mammals. The VPS proteins form distinct functional complexes, which include complexes known as ESCRT, retromer, CORVET, HOPS, GARP, and PI3K-III. Little is known about the orthologs of VPS proteins in insects. Here, with the newly annotated *Manduca sexta* genome, we carried out genomic comparative analysis of VPS proteins in yeast, humans, and 13 sequenced insect genomes representing the Orders Hymenoptera, Diptera, Hemiptera, Phthiraptera, Lepidoptera, and Coleoptera. Amino acid sequence alignments and domain/motif structure analyses reveal that most of the components of ESCRT, retromer, CORVET, HOPS, GARP, and PI3K-III are evolutionarily conserved across yeast, insects, and humans. However, in contrast to the VPS gene expansions observed in the human genome, only four VPS genes (VPS13, VPS16, VPS33, and VPS37) were expanded in the six insect Orders. Additionally, VPS2 was expanded only in species from Phthiraptera, Lepidoptera, and Coleoptera. These studies provide a baseline for understanding the evolution of vesicular trafficking across yeast, insect, and human genomes, and also provide a basis for further addressing specific functional roles of VPS proteins in insects.

1. Introduction

In eukaryotic cells, the endomembrane network consists of morphologically distinct organelles with different functions in cargo sorting and degradation. Constant communication between these organelles is usually through the exchange of trafficking vesicles. Transport of lipids and protein cargos between the intracellular compartments, including transport to and from the plasma membrane, is precisely regulated to maintain the integrity and dynamics of the cell and its organelles (Brighouse et al., 2010; Campelo and Malhotra, 2012).

In the yeast *Saccharomyces cerevisiae*, the vacuole is a prominent organelle that shares functional characteristics with lysosomes from other eukaryotic groups such as vertebrates and invertebrates. The vacuole and lysosomes play vital roles in hydrolysis of proteins, carbohydrates, and lipids. Vacuolar proteins are usually segregated from secretory proteins, and are packaged into transport vesicles and trafficked specifically to the vacuole. Genetic screening studies have identified about 50 vacuolar protein sorting (VPS) genes that affect protein sorting and trafficking to the vacuole in yeast (Arlt et al., 2011; Hedman et al., 2007). Based on the vacuolar morphology observed in cells expressing various VPS mutants, VPS genes were grouped into seven classes, i.e. classes A–F and mutants with tubule-vesicular vacuoles (Banta et al., 1988; Hedman et al., 2007; Raymond et al., 1992). Class A includes VPS8, VPS10, VPS13, VPS29, VPS30, VPS35, VPS38, VPS44, VPS46, VPS51, VPS52, VPS53, VPS54, VPS55, and VPS74. This type of mutant showed wild-type vacuolar morphology, with vacuoles present as 3–10 subcompartments. These proteins may affect the delivery of soluble vacuolar proteins to the vacuole, but have little effect on the

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delivery of membrane proteins to the vacuole. Class B mutants include VPS5, VPS17, VPS39, VPS41, and VPS43, which lack large vacuolar structures and typically contain more than 20 "fragmented" vacuole-like compartments per cell. These small compartments are not functional as targeting sites for the VPS system. Class C VPS mutants contain no identifiable vacuole and the members of this group include VPS11, VPS16, VPS18, VPS33. Dysfunction of these proteins results in the accumulation of abnormal membrane-enclosed structures, which may be a mixture of vesicle-like organelles and lipid droplets. Class D VPS mutants are characterized as defective for vacuolar segregation, and a slightly enlarged vacuole structure. This group includes VPS3, VPS6, VPS9, VPS15, VPS21, VPS34, and VPS45. Class E mutants comprise another large group that includes VPS2, VPS4, VPS20, VPS22, VPS23, VPS24, VPS25, VPS27, VPS28, VPS31, VPS32, VPS36, and VPS37. Mutations in these genes result in the formation of a smaller organelle, distinct from the vacuole, which appears to be an exaggerated form of a prevacuolar compartment (PVC). Finally, a small group of VPS genes have been characterized as Class F, and these include VPS1 and VPS26. Class F mutants usually have a large central vacuole surrounded by class B-like fragmented small vacuolar structures (Banta et al., 1988; Bonangelino et al., 2002; Hedman et al., 2007; Raymond et al., 1992). An abundance of data has shown that VPS proteins function as distinct complexes that are directly or indirectly involved in vesicle formation, transport, tethering, and fusion with target membranes. These complexes include: 1) the endosomal sorting complex required for transport (ESCRT) (Henne et al., 2011; Hurley, 2010; Raiborg and Stenmark, 2009; Rusten et al., 2012; Schuh and Audhya, 2014); 2) the tethering complexes CORVET (class C core vacuole/endosome tethering), HOPS (homotypic fusion and protein sorting) (Balderhaar and Ungermann, 2013; Nickerson et al., 2009; Solinger and Spang, 2013), and GARP (Golgi-associated retrograde protein) (Bonifacino and Hierro, 2011; Brocker et al., 2010); 3) the retrieval complex, Retromer (Attar and Cullen, 2010; Bonifacino and Hurley, 2008; Seaman, 2012); and 4) the class III PI3K (phosphoinositide 3-kinase) complexes (Vanhaesebroeck et al., 2010).

Although most of the above complexes are well-studied in yeast, the detailed mechanisms of their functions in higher eukaryotes remain incompletely understood. Remarkably, the orthologs of these VPS genes in insects have not been identified or studied in any great detail, even though several of these genes have been characterized in Drosophila (Moberg et al., 2005; Vaccari et al., 2009). In the current study, we carried out a comparative genomic analysis to identify and characterize the orthologs of VPS genes in 13 sequenced insect genomes and we compared gene expansions in insects with those in the human genome. In addition we also analyzed and report conservation of signature motifs associated with most VPS protein groups. Combined these studies present a global view of the conservation and divergence of VPS proteins in insects and their relationships to orthologous human protein families.

2. Methods

2.1. Mining insect genomes for VPS genes

VPS genes of the yeast *S. cerevisiae* and the orthologs from the human genome were used for searches of insect genomes using BLASTP and TBLASTN programs. BLAST searches were performed using databases of sequenced insect genomes from 6 Orders, including Hymenoptera (*Apis mellifera*, *Nasonia vitripennis*, and *Harpegnathos saltator*; http://hymenopteragenome.org), Diptera (*Drosophila melanogaster*, *Aedes aegypti*, *Anopheles gambiae*, and *Culex quinquefasciatus*; http://flybase.org and http://www.vectorbase.org), Hemiptera (*Acyrthosiphon pisum*; http://www.aphidbase.com), Phthiraptera (*Pediculus humanus corporis*; http://www.vectorbase.org), Lepidoptera (*Bombyx mori*, *Danaus plexippus*, and *Manduca sexta*; http://silkworm.genomics.org.cn, http://monarchbase.umassmed.edu, and http://agripestbase/manduca, and http://agripestbase/manduca), and Coleoptera (*Tribolium castaneum*; http://beetlebase.org). Specific BLAST searches were also carried out at the National Center for Biotechnology Information (NCBI).

2.2. Alignments and protein structure analysis

Amino acid sequences were aligned using Clustal W2 at the EMBL-EBI server (http://www.ebi.ac.uk/services). Alignments were edited with GeneDoc software (http://www.nrbsc.org/gfx/genedoc/). Protein domain and motif analyses were performed with the web-based tools InterProScan (Jones et al., 2014) at EMBL-EBI (http://www.ebi.ac.uk/interpro), COILS (Lupas et al., 1991) at the ExPASy server (http://www.expasy.org/proteomics), and Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan). Protein molecular weights (MW) and isoelectric points (pI) were calculated with Compute pI/Mw program at the ExPASy server.

2.3. Phylogenetic analysis

The phylogenetic reconstruction was conducted in MEGA6 (Tamura et al., 2013) using the Neighbor-joining statistical method (Saitou and Nei, 1987). The evolutionary distances were computed with the Jones–Taylor–Thornton (JTT) matrix-based method (Jones et al., 1992). The rate variation among amino acid sites was modeled with a gamma distribution. A distance-based bootstrap test with 1000 replicates set was used to analyze the phylogenetic trees (Felsenstein, 1985).

3. Results and discussion

3.1. The ESCRT pathway

The majority of the core components of the ESCRT complexes are from the class E VPS morphological group (Hurley, 2010). The ESCRT machinery was originally found to be required for the maturation of multivesicular bodies (MVBs) and the biogenesis of vacuolar lysosomes. The ESCRT pathway is essential for sorting ubiquitinated membrane proteins into MVBs by deforming the endosomal-limiting membrane inward, followed by scission to release the newly formed vesicles (Hurley, 2010; Hurley and Hanson, 2010). Recent data indicate that, beyond MVB formation, ESCRT complexes have crucial functions in cytokinesis, viral budding, and autophagy (Caballe and Martin-Serrano, 2011; Raiborg and Stenmark, 2009; Rusten et al., 2012; Votteler and Sundquist, 2013). Most recently, it was found that ESCRTs are also necessary for plasma membrane repair (Jimenez et al., 2014). In yeast and humans, the ESCRT pathway is composed of five distinct subcomplexes: ESCRT-0, -I, -II, and -III, and VPS4-Vta1, as well as some accessory proteins, such as Alix (apoptosis-linked gene 2-interacting protein X) (Henne et al., 2011; Hurley, 2010). Our analysis revealed that these components of the ESCRT pathway are highly conserved in yeast, insects, and humans (Table 1, Fig. S1). In addition, some gene expansions that occurred in the human genome lineage are not present across insect genomes, which appear to be generally uniform in their content of core ESCRT pathway complexes.