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Prevacuolar compartment morphology in *vps* mutants of *Saccharomyces cerevisiae*

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Abstract

Over 60 genes have been identified that affect protein sorting to the lysosome-like vacuole in *Saccharomyces cerevisiae*. Cells with mutations in these vacuolar protein sorting (*vps*) genes fall into seven general classes based upon their vacuolar morphology. Class A mutants have a morphologically wild type vacuole, while Class B mutants have a fragmented vacuole. There is no discernable vacuolar structure in Class C mutants. Class D mutants have a slightly enlarged vacuole, but Class E mutants have a normal looking vacuole with an enlarged prevacuolar compartment (PVC), which is analogous to the mammalian late endosome. Class F mutants have a wild type appearing vacuole as well as fragmented vacuolar structures. *vps* mutants have also been found with a tubulo-vesicular vacuole structure. *vps* mutant morphology is pertinent, as mutants of the same class may work together and/or have a block in the same general step in the vacuolar protein sorting pathway. We probed PVC morphology and location microscopically in live cells of several null *vps* mutants using a GFP fusion protein of Nhx1p, an Na^+/H^+ exchanger normally localized to the PVC. We show that cell strains deleted for VPS proteins that have been previously shown to work together, regardless of VPS Class, have the same PVC morphology. Cell strains lacking VPS genes that have not been implicated in the same pathway show different PVC morphologies, even if the mutant strains are in the same VPS Class. These new studies indicate that PVC morphology is another tier of classification that may more accurately identify proteins that function together in vacuolar protein sorting than the original *vps* mutation classes.

Keywords: *Saccharomyces cerevisiae*; Yeast; Prevacuolar compartment; Late endosome; Morphology; *vps* Mutants

1. Introduction

The compartmental nature of eukaryotic cells requires that they contain an efficient mechanism for transporting proteins to and from various intracellular locations. These delivery systems must have a high level of specificity in order to maintain the unique protein composition found in all organelles. The specificity of protein sorting to the lysosome-like vacuole of bakers' yeast, *Saccharomyces cerevisiae*, is no exception. Unique cellular components must recognize vacuolar proteins, sort them away from secretory proteins, package them into transport vesicles, and deliver them to the vacuole via a prevacuolar compartment (PVC, equivalent to the mammalian late endosome). Many of the molecular mechanisms involved in these delivery pathways are not understood. Several genetic selections have uncovered a large set of yeast mutants that are defective in the vacuolar protein delivery pathway. Most of the gene products affected in these *vps* (vacuolar protein sorting) mutants are part of the trans-acting cellular machinery that is required for sorting proteins to the vacuole. However, a few mutants in *vps* proteins have a less direct effect on protein sorting to the yeast vacuole; these include mutants that are involved in the maintenance of the protein composition of the

Abbreviations: DIC, differential interference contrast; FITC, fluorescein isothiocyanate; PVC, prevacuolar compartment; VPS, vacuolar protein sorting.

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PVC, by serving in the recycling of proteins via vesicle intermediates from the PVC back to the Golgi (Bowers and Stevens, 2005). Nonetheless, all of the *vps* mutants identified have an aberrantly functioning vacuole, and it is crucial to understand the molecular basis for this defective organelle in the different *vps* mutants.

The yeast vacuole, which is the equivalent of the mammalian lysosome, is an acidic compartment involved in macromolecular turnover (Klionsky et al., 1990). Vacuolar hydrolases transit the early stages of the secretory pathway en route to the vacuole/lysosome. In a late Golgi compartment, these proteins are actively sorted away from the pool of secretory proteins and are targeted to the vacuole/lysosome directly or via a PVC (endosomal) intermediate, depending on the proteinaceous cargo. For many vacuolar proteases, the arrival in the vacuole is followed by a proteolytic processing event that activates the hydrolase (Bryant and Stevens, 1998; Klionsky et al., 1990; Vida et al., 1993).

Vacuolar proteases transit the early part of the secretory pathway until they reach the late Golgi where they are actively sorted away from the bulk of the proteins, destined for secretion (Vida et al., 1993). Alteration of the vacuolar sorting of several proteases results in its appearance at the cell surface (Bankaitis et al., 1986; Robinson et al., 1988; Rothman et al., 1989; Rothman and Stevens, 1986). This mislocalization and subsequent secretion of vacuolar proteins are the basis of several genetic selections that have resulted in the isolation of a large number of mutants defining over 40 complementation groups specifically defective in the delivery of proteins to the vacuole (Robinson et al., 1988; Rothman and Stevens, 1986). In addition, other selections and screens have identified additional mutants that are defective in vacuolar protein sorting (Avaro et al., 2002; Bonangelino et al., 2002; Entian et al., 1999). Together these mutant collections describe over 60 different proteins involved in the VPS pathway (Bowers and Stevens, 2005).

In order to gain a better understanding of the cellular processes affected in the vacuolar protein sorting mutants, morphological studies of the vacuole were carried out. The *vps* mutants were found to fall into seven morphological classes, Classes A–F and mutants with tubulo-vesicular vacuoles (Banta et al., 1988; Conibear et al., 2003; Horazdovsky et al., 1994; Kucharczyk and Rytka, 2001; Raymond et al., 1992). Class A mutants contain a wild type appearing vacuole, which consisted of 1–3 prominent structures, which in some cases were interconnected. Class A mutants, therefore, appear to affect the delivery of soluble vacuolar proteins and have little effect on the delivery of membrane to the vacuole. The other classes of *vps* mutants also have a vacuole that functions at less than wild type capacity and show more severe and distinct morphological defects than Class A mutants. The mutants that comprise the Class B morphology group lack any large vacuolar structure. Instead, these mutants contain about 30–40 small vacuole-like structures. These small structures are acidic, but are not completely competent as targets for the vacuolar protein sorting system. Class C *vps* mutants show the most pronounced morphological defect; they have no identifiable vacuolar structure but do accumulate many abnormal membrane enclosed structures. Class D mutants are characterized by the presence of a slightly enlarged vacuole structure, defects in mother to daughter vacuole inheritance, and an improperly assembled vacuolar H^+-ATPase. Class E *vps* mutants accumulate a novel organelle distinct from the vacuole. This structure is smaller than the vacuole, is acidic, and contains vacuolar ATPase but not the vacuolar membrane protein alkaline phosphatase (ALP). The Class E structure is thought to represent an exaggerated form of the PVC. Like the Class B mutants, Class F mutants contain small fragmented vacuole-like structures, but they also contain a large vacuole as well. Tubulo-vesicular vacuoles, different in structure than the original morphological classes, have also been seen in several *vps* mutants (Conibear et al., 2003; Conibear and Stevens, 2000).

This initial characterization of the *vps* mutants has had its limitations, however. Several *vps* mutant classes describe a set of trans-activating factors that function at the same point in the lysosomal protein targeting pathway, such as the Class C VPS proteins and subsets of Class E proteins (Bowers and Stevens, 2005). However, the Class A VPS proteins function at several disparate places in the vacuolar pathway. For example, Vps30p functions in autophagy and protein recycling from the PVC to the Golgi (Kametaka et al., 1998; Kihara et al., 2001; Seaman et al., 1997). Vps29p and Vps35p function with the Class B proteins Vps5p and Vps17p and a Class F protein Vps26p to recycle CPY receptor from the PVC to the Golgi, in a protein complex termed the retromer (Seaman et al., 1998). Vps55p functions to traffic proteins from the PVC to the vacuole and also has a defect in endocytosis (Belgareh-Touzé et al., 2002). We feel that the initial classification of the VPS proteins placed many into apparent functional categories that turned out to be incorrect or simplistic for revealing the myriad of pathways of protein sorting to the yeast vacuole.

The focus of this research is to begin to extend this initial classification by studying the structure and subcellular location of the PVC in several *vps* mutants, using a GFP fusion protein of a *bona fide* PVC protein, Nhx1p, which is an Na^+/H^+ exchanger on the PVC membrane (Nass and Rao, 1998). The PVC of *S. cerevisiae* is a membrane-bound compartment, usually juxtaposed next to the vacuole (Gerrard et al., 2000; Odorizzi et al., 2003; Piper et al., 1995). One of the main functions of the PVC is as a last protein sorting point before the proteins are either sent onto the vacuole or recycled back into the early secretory pathway, reviewed in Bowers and Stevens (2005). This organelle is characterized by its ion balance, and this ion balance is required for proper vacuolar protein sorting out of the PVC (Bowers et al., 2000; Brett et al., 2005). One ion transporter that is required for PVC ion balance is the $Na^+(K^+)/H^+$ exchanger, Nhx1p (Bowers et al., 2000; Brett et al., 2005; Nass and Rao, 1998). This sodium/proton exchanger is a PVC integral membrane protein (Nass and Rao, 1998), required for proper sorting from the PVC to the vacuole (Bowers et al., 2000). We used a GFP tagged version of Nhx1p (kind gift of Dr. Rajini. Rao, Johns Hopkins University) that has previously been shown to