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Implication of Yeast Dynamin-Related Protein Vps1 in Endocytosis and Organelle Fission

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ABSTRACT

Dynamin is a multi-domain GTPase found in mammals, which plays an imperative role in fission events at the plasma membrane during endocytosis and membrane-bound internal organelles, including the Golgi membrane (Ramachandran 2011). It has been shown that dynamin and its yeast counterpart, vacuolar protein sorting 1 (Vps1), are highly related proteins sharing similar sequence and function. Through this in-depth review of the literature, we summarize main functions of the dynamic Vps1 protein at diverse locations within the cell.

Keywords: *dynamin, Vps1, endocytosis, Golgi, scission*

1. BACKGROUND

Vps1 is a GTP-binding (GTPase) protein found in the budding yeast, *Saccharomyces cerevisiae*, and is homologous to the mammalian protein dynamin (van der Bliek 1999). Dynamin is known to be involved in the endocytic process as well as other membrane scission and fusion events (Ramachandran 2011, Schmid and Frolov 2011). Endocytosis involves the uptake of macromolecules into the cell. Following this process, protein trafficking and recycling occurs, which allows the transport of nutrients and waste throughout cellular compartments. Defects in these essential functions and pathways can lead to disease. For example, the low-density lipoprotein receptor (LDLR) is a cell surface glycoprotein that plays a critical role in controlling blood cholesterol level by uptaking cholesterol-containing lipoprotein particles from circulation (Jeon and Blacklow 2005) via endocytosis. Mutations to the LDLR are associated with Familial Hypercholesterolemia (FH), an inherited genetic disease, affecting ~1 in 500 people in most populations. FH is characterized clinically by a lifelong elevation of LDL in blood and is associated with an increased risk of coronary artery disease and cardiovascular diseases. It is also known that endocytic defect of the macrophage is associated with atherosclerosis (Schrijvers et al. 2005). Interestingly, the budding yeast *Saccharomyces cerevisiae* internalizes membrane receptor proteins and lipids from the plasma membrane in the same way (Engqvist-Goldstein and Drubin 2003), as higher eukaryotes do. Given this, investigating the precise roles of dynamin and its homolog, Vps1, on endocytic processes as well as other trafficking could potentially shed some light on the mechanisms of those diseases and conditions affecting human health.

2. Vps1 IN ENDOCYTOSIS

Endocytosis is an essential process by which cell engulfs molecules that are either essential to the cell's function or need to be sent to the vacuole for degradation. Yeast dynamin like protein Vps1, similar to its mammalian counterpart, has been thought to share this significant role in endocytosis, due to sharing a high degree of amino acid homology with dynamin (Vater et al. 1992). In 2004, Yu and Cai published an article (Yu and Cai 2004), which showed that Vps1 is necessary for the proper organization of actin cytoskeleton, based on the finding that the loss of Vps1 led to the formation of an aberrant actin cytoskeleton, exhibiting "depolarized and aggregated structures". Most importantly, it appears that the aberrant actin organization severely affects the efficiency in the internalization of membrane receptors such as Ste3 via endocytosis. In order to further examine the precise role of Vps1 in endocytosis, the authors investigated a possible functional relationship between Vps1 and the well-known endocytic adaptor protein Sla1. Sla1 is a protein known for its role in the assembly of actin cytoskeleton and its interaction with various proteins related to the invagination process (Warren et al. 2002). The authors demonstrated that Sla1 and Vps1 partially colocalize at endocytic sites via the physical interaction between them. Taken together, they proposed a model for Vps1 in which it functions for the proper organization of the actin cytoskeleton, which is considered to be essentially required for efficient endocytosis, via its interaction with Sla1.

A recent study published in the European Journal of Cell Biology in 2010 demonstrated that *vps1* mutant cells exhibited an abnormally increased lifespan of newly forming cortical endocytic vesicles carrying Las17-GFP, Ede1-GFP, Sla1-GFP, and Abp1-GFP (Nannapaneni et al. 2010). The most significant finding in this research

relative to the endocytic process was that the loss of Vps1 led to an aberration in endocytic internalization dynamics, manifested by a slower motility during endocytic vesicle internalization. For example, Abp1 (actin binding protein 1), a known marker for endocytic sites, is recruited to the endocytic site and then internalizes rapidly toward the cytoplasm, moving in a directed manner in a normal cell. However, Vps1 deficient cells exhibited a slow, random motion of Abp1.

Given that protein localization is tightly linked with function, a group of scientists studied the subcellular localization of Vps1 and found that RFP-tagged endocytic markers such as Sla1-mRFP and Abp1-mRFP are partially colocalized with Vps1-GFP (Smaczynska-de et al. 2010). According to their spatiotemporal analysis regarding the recruitment of Vps1 to the endocytic site, Vps1 is recruited to the endocytic site slightly after Abp1 is recruited, at a time compatible with the beginning of invagination. This lab also discovered that upon *VPS1* deletion, the lifespan of five different endocytic proteins at endocytic sites were increased and the invagination process was abnormal and attenuated, which is consistent with the published data by Nannapaneni et al (2010). Their ultra-structural study with electron microscopy showed that "pronounced invagination" is dramatically decreased (24%) in *vps1*Δ cells and that the angle of invagination is also increased, leading to abnormal scission, or pinching-off of the plasma membrane.

Amphiphysin protein Rvs167 was thought to be linked to endocytosis due to their ability to remodel the membrane (Douglas et al. 2009, Ren et al. 2006). Indeed, a study published in the *Journal of Cell Science* in 2010 showed that the double mutant cells lacking both *VPS1* and *RVS167* were synthetic lethal at 37 degrees Celsius, suggesting a genetic interaction between the two genes and a potential physical interaction *in vivo* (Smaczynska-de et al. 2010). Later, the authors, using a bimolecular fluorescence complementation assay where a detectable fluorescence appears as two proteins physically interact with each other, revealed a physical interaction between the two proteins (Smaczynska-de et al. 2012). It is known that Vps1 consists of three 'domains'; the N-terminal GTPase domain, the middle domain, and the C terminal region (GTPase effector). Naturally, the question of which domain of Vps1 is implicated in the binding of Rvs167 arose. Given the fact that Rvs167 contains SH3 domain and that the domain binds to a proline-rich region of other proteins, the authors hypothesized that the central proline rich domain of Vps1 might interact with the SH3 domain of Rvs167. This hypothesis according to their yeast two hybrid assay was proven correct. Furthermore, a systemic mutation analysis revealed the precise amino acid that binds Rvs167; proline 564 in Vps1, located in front of the GTPase effector domain. A replacement mutation replacing the proline with alanine abolished the interaction between Vps1 mutant and intact Rvs167. As a result, mutant cells expressing the mutant version of Vps1 exhibited a severe endocytic defect, manifested by numerous abnormally elongated membrane invaginations at the plasma membrane (Mishra et al. 2011, Smaczynska-de et al. 2012). Based on these observations, one can conclude that the *in vivo* interaction between Vps1 and Rvs167 is essentially required for their functional cooperation in membrane scission.

3. Vps1 IN INTRACELLULAR TRAFFICKING AND REMODELING OF THE MEMBRANE

3.1 Implicated in Vacuolar Protein Sorting and Targeting at the Golgi

Vps1 was originally called Vpl1 (vacuolar protein localization 1) and sometime later, Vpt26 (vacuolar protein targeting 26) (Banta et al. 1988, Rothman and Stevens 1986). In 1986, a research group discovered that cells lacking this specific protein were unsuccessful in transporting a vacuolar luminal enzyme, carboxypeptidase Y (CPY), to the vacuole (Rothman and Stevens 1986). This finding led to the protein's current name, vacuolar protein sorting, or Vps1. In a normal yeast cell, CPY is created in the endoplasmic reticulum and is then sent to the Golgi complex for maturation (Deitz et al. 2000, Muller and Muller 1981). After CPY is converted to its mature form, mCPY, it makes its way to the late endosome and then to the vacuole where it assists in degrading proteins. In *vps*1 null cells, the functional CPY pathway is redirected from the Golgi to the outside of the cell via an intact exocytosis (Rothman and Stevens 1986), suggesting Vps1 plays a vital role in transporting the CPY to the late endosome. An important protein necessary for successful CPY delivery is Vps10. Vps10 is a transmembrane protein that serves as a CPY receptor for transport to the pre-vacuolar endosome (Marcusson et al. 1994). It is postulated that the Vps10 mechanism involves binding CPY at the Golgi and traveling to the endosome, where it then disassociates from CPY and is recycled back to the Golgi for future CPY uptake and transportation (Marcusson et al. 1994, Van Dyck et al. 1992). Nothwehr and Stevens further found that in *vps1* mutant cells there is an accumulation of Vps10 at the vacuolar membrane, which might indicate there is not only a blocked pathway from the Golgi to the endosome, but also from the endosome back to the Golgi (Nothwehr et al. 1995). Another Golgi membrane protein, Kex2, is also disrupted in *vps1* null cells. Rather than being present in vesicles destined for the prevacuole as it should, Kex2 is redirected to the plasma membrane where it is then endocytosed and moved to the vacuole (Conibear and Stevens 1995, Nothwehr et al. 1995, Wilsbach and Payne 1993). The missorting and redirection of Golgi proteins left important lingering questions that have been driving forces for a great deal of Vps1 research in the last 25 years.

3.2 Vps1 Functions with Clathrin

Clathrin is a coat protein that plays an imperative role in the formation of invaginating vesicles at the plasma membrane and the Golgi (Mooren et al. 2012). The appropriate formation and scission of these clathrin-coated vesicles is vital for functional protein transport. Knowing that Vps1 functions in vacuole protein sorting and targeting, a group of researchers performed a growth assay using a combined mutation of a Vps1 (*vps1*^{ts}) and a clathrin heavy chain (*chc1-521*), and they found that the double mutant cells experienced a severe synthetic sickness, indicating a functional relationship between the two proteins (Bensen et al. 2000). The double mutant cells with the *chc1-521* and *vps1*^{ts} alleles were also found to harbor an aberration in alpha factor maturation. Because the maturation of alpha factor heavily relies on the proper functioning of Kex2, it may be that the observed maturation defect in the mutant cells is due to a mistargeting of Kex2 to the plasma membrane. It is important to note that an appropriate membrane remodeling occurring at the Golgi is a prerequisite for proper sorting and targeting of CPY to the late endosome and then to the vacuole. It has been widely accepted that the budding of vesicles from the late Golgi, targeted toward the prevacuolar compartment (PVC), involves not only clathrin but also another proteins, such as Vps34 and Vps15. Vps34 is a Golgi-associated lipid kinase that shows similarities to the catalytic subunit of the mammalian phosphoinositide 3-kinase (PI 3-kinase), p85/p110 that phosphorylates phosphatidylinositol (Ptdlns) to form Ptdlns(3)P (Stephens et al. 1991). Vps15, a serine/threonine protein kinase, has been characterized to activate and recruit Vps34 to the Golgi membrane, where it can bind to its lipid substrates (Stack et al. 1995). Noteworthy, Ptdlns(3)P synthesis has been correlated with the ability of budding yeast cells to transport proteins from the Golgi to the vacuole (Stack et al. 1995). Moreover, it has been proposed that the Vps15-Vps34 heterodimer directly binds to Vps10-CPY complex to facilitate CPY sorting into the clathrin-coated vesicle at the late Golgi (Conibear and Stevens 1995). Vps1 may then act to mediate the pinching off of the clathrin-coated vesicle. In this view, Vps34 is not required for actual vesicle formation, but instead is required for the sorting of vesicle cargo.

3.3 Vps1 in Peroxisome and Vacuole Scission

Vps1's function has been proven to be incredibly multifaceted. Thus far, the review has mentioned its secretory roles at the Golgi, and at the plasma membrane in endocytosis. Recent research has indicated Vps1's role in peroxisomal homeostasis. Peroxisomes are membrane bound organelles, which function in the catabolism of fatty acid chains. Peroxisomes contain enzymes that play significant roles in energy metabolism. Proper peroxisomal functioning is imperative for mammalian health; loss of peroxisomal function leads to cause of diseases, ranging from relatively mild single enzyme deficiencies to severe syndrome (Hoepfner et al. 2001, Purdue and Lazarow 1994). Research has shown that in Vps1 null cells, there are only one or two large peroxisomal masses rather than multiple, smaller peroxisomes, suggesting that Vps1 is responsible for an important aspect of membrane fission in the peroxisome (Hoepfner et al. 2001). It has also been noted that Vps1 associates with peroxisomes in a Pex19-dependent manner (Vizeacoumar et al. 2006). Pex19 is a peroxin that acts as a shuttling receptor for peroxisomal membrane proteins or as a chaperone assisting the assembly/stabilization of proteins at the peroxisomal membrane (Vizeacoumar et al. 2006). Vps1 contains two Pex19 recognition sequences at amino acids 509-523 and 633-647 (Vizeacoumar et al. 2006). Deletion of the first sequence, but not the second, results in a reduction in numbers of peroxisomes, as seen in *vps1*Δ cells (Vizeacoumar et al. 2006). Using yeast two-hybrid analysis, Vizeacoumar and colleagues also found that Pex19 and Vps1 interact. This strengthened their idea that Pex19 acts as a chaperone to stabilize the association of Vps1 with peroxisomes (Vizeacoumar et al. 2006). It is therefore possible that Pex19 recruits Vps1 to the peroxisomal membrane for a scission event to occur. The mechanism of Vps1 recruitment is not currently understood, which indicates the need for future research in this area. Vps1's homolog, Dnm1, also had an effect on peroxisomes. Deletion of Dnm1 was shown to reduce the number of peroxisomes, and when deleted along with Vps1, there was a more severe phenotype with cells containing only one large peroxisome (Kuravi et al. 2006). Membrane fission and fusion events are critical for maintaining cellular homeostasis. The dynamic protein Vps1 has also been shown to mediate vacuole fission, in addition to the aforementioned scission roles elsewhere in the cell (Peters et al. 2004). According to the model proposed by Peters et al (2004), to promote vacuole scission, Vps1 that is localized at the vacuole membrane binds to Vam3 protein, a component of vacuolar SNARE complex, via its GTPase effector domain (GED). The binding of Vps1 to Vam3 silences fusion activity long enough for the scission mediated by Vps1 to be completed. Once scission is accomplished, Sec18 disassembles Vps1 and ceases fission activity, while also stimulating Vam3 for fusion. In the absence of Vps1, it was shown that fission and fusion are impaired due to the lack of SNARE mediation and organization, which ultimately disrupts vacuolar homeostatic balance.

4. CONCLUSION

This review reveals Vps1 to be exceptionally multifunctional and multi-localized at the plasma membrane and within the cell. Beside its localization at vacuole and endocytic sites, our lab has recently found that Vps1 coincides with endosomes and the late Golgi (Data not published), which further strengthens our notion of Vps1 being a dynamic multifunctional protein.

Regardless of its localization, all the Vps1 studies propose one common function of Vps1, namely membrane scission at various membrane-bound compartments. Accordingly, Vps1 works collectively with different partners at the plasma membrane (Sla1 and Rvs167), the Golgi (Clathrin), peroxisomes (Pex19), and the vacuole (SNARE complex) to perform similar tasks imperative for healthy cellular function and transportation. The detail mechanism by which Vps1 is recruited to the specific working sites and plays collaborative jobs together with the aforementioned proteins remains to be explored in order to advance contemporary understanding of the multifaceted Vps1 protein.

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