Review Article

Biogenesis and Function of Late Endosomes and Lysosomes

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Lysosomes are membrane bound organelles that mainly serve as sites for degradation in the cell. Studies in a variety of model systems have provided insights about organization and functions of late endosomes and lysosomes. Molecular players involved in biogenesis, regulation and maintenance of lysosomes seem to be conserved across different model systems from yeast to mammals, signifying fundamental roles these organelles play in all eukaryotic cells. The role of lysosomes in controlling the duration of cell signaling and thus sculpting a morphogenetic gradient is critical in the developmental plan of a metazoan. In this review, we synthesize studies on organization, composition, and mechanism of biogenesis of late endosomes and lysosomes to provide insights into their function in higher metazoa and yeast.

Key Words: Endocytosis, multi-vesicular bodies, lysosomes, maturation, signaling

Introduction

Eukaryotic cells are characterized by the presence of numerous functionally distinct membrane-enclosed organelles. Of these, lysosomes are major degradative organelles that serve as the terminal destination for many endocytic, autophagic and secretory materials targeted for destruction in the cell.

Endocytosis is a process of internalization of extracellular fluid or cell surface receptors via diverse cell surface invaginations, generating an endosomal membrane system (Conner and Schmid 2003, Gruenberg, 2001, Gruenberg and Maxfield 1995, Guha et al. 2003, Mellman et al. 1986, Mukherjee et al. 1997, Sharma et al. 2002). It plays an important role in uptake of nutrients, scavenging of extracellular material, internalization of receptor bound ligands such as growth factors, hormones, lipoproteins and antibodies (Mellman et al. 1986, Mukherjee et al. 1997). Following internalization macromolecules are located in membrane bound vesicles referred to as endosomes.

Subcompartments of the endosomal system

The endosomal system may be thought to consist of membrane compartments, categorized as 'early' or 'late' based on the kinetics with which endocytosed material accesses these compartments, specific endosomal protein composition and extent of endosomal acidification (Fig. 1). The early endosomal system comprises a heterogeneous population of dynamic and acidic endosomes, distributed towards the cell periphery. These are compartments accessed by macromolecules immediately after internalization via endocytic vesicles directly derived from the cell surface. They are capable of fusion with each other. They also serve as the main

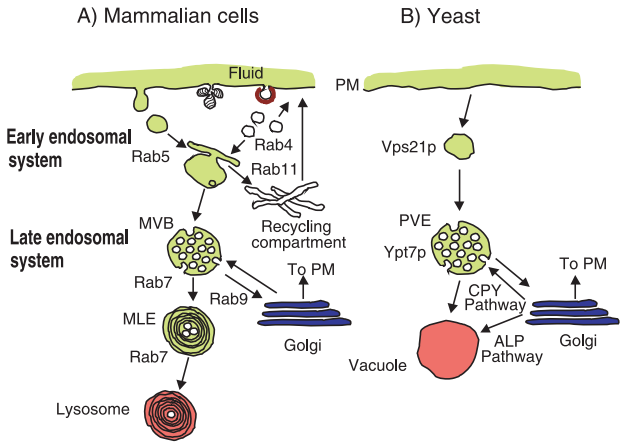

Figure 1: *In mammalian cells, cell surface membrane proteins and solutes are endocytosed via multiple pathways and delivered to Rab5 positive early endosomes. Many macromolecules are rapidly recycled back to the plasma membrane from sorting early endosomes either directly or via the endocytic recycling compartment. In contrast, other membrane proteins that include receptors targeted for down regulation, ligands and the remaining solutes are trafficked to Rab7 positive late endosomes and lysosomes. Late endosomes are multi-vesicular (MVE) or multi-lamellar (MLE) compartments capable of fusion with vesicles in the biosynthetic pathway from* trans*-Golgi network (TGN). Newly synthesized lysosomal enzymes bound to mannose-6-phosphate receptors (M6PRs) are delivered from TGN to endosomes and then routed towards lysosomes, whereas M6PRs are recycled back to TGN, a process regulated by Rab9. In yeast, Rab5 homologue Vps21p localizes to early endosomes that receive endocytosed cargo immediately post internalization. The pre-vacuolar endosome (PVE) is multi-vesicular and is accessed by both endocytosed cargo as well as biosynthetic cargo from TGN. PVE fuses with the vacuole delivering its contents to the vacuole. Cargo destined for the vacuole from TGN is delivered via two distinct pathways in yeast. Carboxypeptidase Y (CPY) is delivered to PVE prior to vacuole unlike alkaline phosphatase (ALP) that is directly delivered to the vacuole.*

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sites for sorting of endocytosed material and membrane towards lysosomal degradation or recycling to the cell surface (Mukherjee et al. 1997).

The late endo-lysosomal system comprises of two types of endosomal compartments acidic pre-lysosomes or late endosomes, and lysosomes. The acidic pre-lysosome and late endosomes receive cargo directly from the early endosomal sysem and acts as another waystation for molecules enroute to lysosomes. This is also a dynamic system of membrane organelles of diverse morphology [Fig. 1 (Griffiths et al. 1988, Ohkuma and Poole 1978, Schmid et al. 1988)]. These membrane compartments are devoid of recycling receptors such as the transferrin receptor (Luzio et al. 2000). Late endosomes are found mostly near microtubule-organizing centers in the cell and appear multi-vesicular or multi-lamellar (Kobayashi et al. 1998). They fuse with lysosomes in addition to being capable of fusion with vesicles derived from *Trans*-Golgi network (TGN) via the biosynthetic pathway (Luzio et al. 2000, Mullins and Bonifacino 2001).

Experimental observations drawn from a variety of model systems including *Saccharomyces, Drosophila, Mus musculus* have provided insights about organization, composition and function of late endosomes and lysosomes. Studies in yeast have identified the vacuole to be equivalent of lysosome that receives endocytic cargo as well as biosynthetic cargo from the Golgi (Fig. 1). Late endosomal system in yeast is constituted by pre-vacuolar multi-vesicular endosome (PVE) and vacuole (Fig. 1). Genetic screens in yeast have isolated numerous candidate molecules that set up the endosomal and vacuolar system (Banta et al. 1988, Robinson et al. 1988).

The Rab protein paradigm

Molecular players responsible for regulation and maintenance of all membrane compartments including late endo-lysosomal system are conserved from yeast to mammals, highlighting the importance and evolutionary relatedness of this system of organelles in all eukaryotic cells. The Rab proteins are key players. These are prenylated monomeric, small molecular weight GTPases of the Ras superfamily involved in regulation of membrane trafficking in all eukaryotes (Zerial and McBride, 2001). A number of different Rab proteins are involved and specifically associated with the different elements of the endosomal system [Fig. 1 (Chavrier et al. 1990, Christoforidis et al. 1999)]. Cytosolic Rab5 associates specifically with early endosomal membranes and regulates early endosomal homotypic fusion. Compared to Rab5 positive early endosomes, late endosomes associate with Rab7 and Rab9 (Chavrier et al. 1990). Rab7 function has been implicated in early endosome to late endosome membrane trafficking while Rab9 function has been implicated in vesicular trafficking between the late endosome and TGN (Barbero et al. 2002, Chavrier et al. 1990). In yeast, molecular players such as Vps21p, yeast homologue of Rab5, regulate the early endosomal system that is not as elaborate as observed in higher metazoa. Analogous to Rab7 function in late endosomal system in higher metazoa, the yeast homologue of Rab7, Ypt7p has been shown to be involved in vacuolar membrane trafficking.

For a discussion on the early endosomal system see other reviews (Mills et al. 1999, Pfeffer, 2003). In this review, studies on the organization, composition, mechanism of formation of late endosomes and lysosomes, and their function in higher metazoa and yeast will be discussed.

Organization, composition and biogenesis of late-endosomal system

Late endosomes are large (0.2-1µm in diameter) spherical juxtanuclear compartments (van Deurs et al. 1993), rather unlike early endosomes which are tubular vesicular structures of variable size and morphology (Gruenberg, 2001). These have been categorized based on morphology at the electron microscopic (EM) level as multi-vesicular or multi-lamellar compartments (Kobayashi et al. 1998, Murk et al. 2002).

Multi-vesicular late endosomes

Multi-vesicular late endosomes (MVBs) are characterized by a variable number of internal vesicles 50-70 nm in size within the limiting endosomal membrane as observed using EM in diverse cell types (Anderson et al. 1984, Piper and Luzio 2001, Sriram et al. 2003). They have been described as pre-lysosomal acidic compartments involved in delivery of endocytic and autophagic material to lysosomes for destruction. Biochemical fractionation of late endosomes from mammalian cells followed by analysis of lipid composition indicates that MVBs are rich in triglycerides, cholesterol esters and an unusual phospholipid, lysobisphosphatidic acid [LBPA, (Kobayashi et al. 1998)]. In addition to phosphatidylcholine and phosphatidyl ethanolamine that accounts for 68 % of phospholipids, LBPA and phosphatidyl inositol are the major phospholipids present in MVB [14 % and 8 % respectively, (Kobayashi et al. 1998)].

MVBs are thought to be formed from early sorting endosomes that have sorted away macromolecules intended to be recycled such as the transferrin receptor, LDL receptor, and most membrane components (Geuze, 1998, Mayor et al. 1993) (Gruenberg and Maxfield 1995, Raiborg et al. 2003). Mechanisms for their formation still remain controversion (see section 5.1). Multi-vesicular morphology of endosomes is a result of the

inward budding of sorting endosomal membranes forming intra-endosomal vesicles that contain cytosol in their lumen (Fig. 2). This seems to be a tightly regulated two-step process (Fig. 2). In the first step, endosomal phosphatidylinositol 3-phosphate (PI3P) recruits cytosolic proteins that assemble bilayered clathrin coats on early endosomes. Subsequently, in a second step the endosomal membrane buds inwards at sites adjacent to bilayered clathrin coats. The mechanism by which inward budding of endosomal membranes occur is poorly understood. PI3P has been observed on limiting endosomal membrane as well as some lumenal vesicles of MVB (Gillooly et al. 2000). Reducing endosomal PI3P levels in Mel JuSo or epithelial cells, Hep-2 using PI3-Kinase inhibitor Wortmannin prevents formation of MVBs (Fernandez-Borja et al. 1999, Futter et al. 2001) suggesting a crucial role for PI3Ps in multivesicularization.

LBPA, predicted to be an inverted cone shaped lipid that induces membrane curvature (Gruenberg, 2001, Matsuo et al. 2004), also regulates endosomal multi-vesicularization. It has been observed that LBPA is heavily enriched in internal vesicles of MVBs compared to the limiting endosomal membrane (Kobayashi et al. 1998). LBPA regulates sorting of proteins such as mannose-6-phosphate receptor (M6PR) in MVB and their recycling back to TGN from MVB (Kobayashi et al. 1998). M6PR is a multi-functional receptor for lysosomal enzymes bearing mannose-6-phosphate modification. Newly synthesized lysosomal enzymes such as cathepsin D that acquire a mannose-6-phosphate modification in TGN are delivered via the biosynthetic pathway to endosomes by M6PR. Post endosomal delivery, M6PR is trafficked back to TGN to get reutilized (Ghosh et al. 2003). Endocytosis of LBPA antibody into late endosomes prevents recycling of M6PR from MVB back to TGN and effectively mis-localizes M6PR from TGN to late endosomes in BHK cells (Kobayashi et al. 1998). Thus LBPA seems to be functionally essential for recycling of cargo back from MVB to TGN.

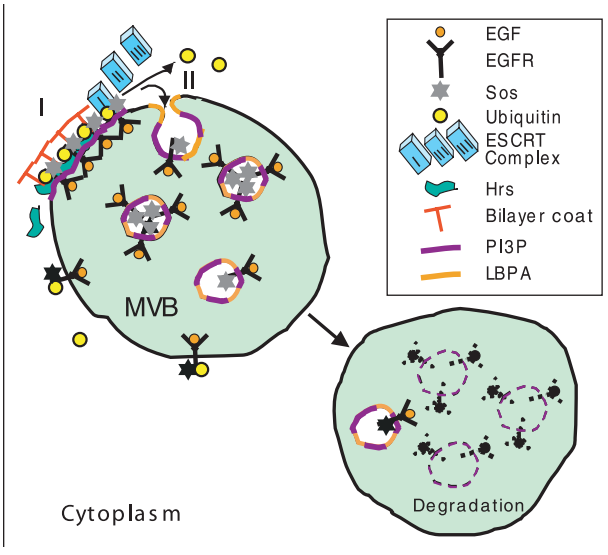

Figure 2: *Schematic depicts the two steps likely to be involved in the formation of MVB from early sorting endosomes. PI3P synthesized on endosomes recruits bilayered clathrin coats via cytosolic proteins such as Hrs (Step I). These bilayered clathrin coats are likely to play a role in sequestration of ubiqitinated signaling receptors. Subsequently, LBPA is involved in inward budding of endosomes resulting in multi-vesicularization (Step II). Multi-vesicularization results in sorting of endocytosed signaling receptors into the intralumenal vesicles. Endocytosed growth factor receptors such as EGFR remain signaling competent in endosomal compartments. They recruit downstream signal transducers such as Sos and other cytosolic effectors for this purpose. Hrs recruits and sorts mono-ubiquitinated receptor via multiple protein complexes (ESCRTs) into lumenal vesicles of MVB resulting in abrogation of signaling, as the receptor bound cytosolic signal transduction machinery remains no longer signal transduction competent. The lumenal vesicles of the MVB and their contents are subsequently degraded.*

MVBs formed from early endosomes are highly dynamic in structure and composition. During multi-vesicularization of early endosomes, endosomes move from the cell periphery towards the pericentriolar region on microtubules (Pastan and Willingham, 1981) using dynein, a minus-end-directed cytoplasmic motor (Bomsel et al. 1990, Harada et al. 1998, Wubbolts et al. 1999). Incubation of Hep2 cells with Horse-radish peroxidase (HRP) for 2.5-30 min results in delivery of endocytosed HRP to MVBs (van Deurs et al. 1993). The number of lumenal vesicles in MVBs increases with time; early MVBs have few internal vesicles and contain transferrin receptor while late MVBs contain numerous internal vesicles and are devoid of transferrin receptor (van Deurs et al. 1993). In addition to acquiring endocytic cargo, MVBs also fuse with Golgi derived vesicles carrying biosynthetic cargo (Mullins and Bonifacino, 2001, Piper and Luzio 2001).

MVBs are involved in cellular cholesterol homeostasis (Kobayashi et al. 1999, Mobius et al. 2003). Excess cholesterol accumulation in MVBs affects late endosome membrane trafficking of sphingolipids as seen in cells from Niemann-Pick type C1 patients [NPC 1, (Puri et al. 2003)]. Cholesterol recruits annexin-II, a cholesterol binding protein to MVBs. Annexin II has been shown to be involved in MVB formation (Mayran et al. 2003). Thus, PI3P, LBPA and cholesterol play a structural as well as sorting role in MVB in higher metazoa.

In yeast strains, EM studies have not revealed late endosomal MVBs either due to technical problems associated with preservation of late endosomal compartment or presence of very robust lipases involved in degradation of lumenal lipid vesicles. However, in mutants of yeast that show reduced vacuolar hydrolase activity (*vma4*Δ), lumenal vesicles in vacuoles have been

observed suggesting a multi-vesicular intermediate prior to vacuole formation (Morano and Klionsky, 1994).

In contrast to mammalian cells, LBPA has not yet been isolated in yeast despite the proposal of a fundamental role for LBPA in recycling membrane proteins from late endosomes to TGN in higher metazoa (Piper and Luzio, 2001). PI3P has been found in intra-endosomal vesicles of vacuoles and synthesis of phosphatidyl-3,5-bis-phosphate from PI3P on endosomal membrane by PI3-Kinase Fab1p has been shown to be essential for multi-vesicularization of pre-vacuolar endosomes (PVE) and sorting of membrane protein carboxypeptidase S into PVE (Katzmann et al. 2002, Odorizzi et al. 1998a, Wurmser and Emr 1998).

MVBs are rich in highly glycosylated membrane proteins that are delivered via the endocytic pathway or biosynthetic pathway (Mullins and Bonifacino, 2001, Piper and Luzio 2001). These membrane proteins are differentially distributed between lumenal vesicles and limiting endosomal membrane as indicated in table 1 (Katzmann et al. 2002, Storrie 1988). The vacuolar proton pump (V-type H^+-ATPase) and glycoproteins such as lysosome associated membrane protein 1 (LAMP1) and LAMP2 are associated with the limiting membrane of MVB where they constitute more than 50 % of total membrane proteins (Marsh et al. 1987).

Table 1:

Limiting membrane	Lumenal vesicles
	Plasma membrane
	Transmembrane receptors
Yeast	Yeast
V-ATPase	Ste2p
Dipeptidyl aminopeptidase B	Ste3p
Alkaline phosphatase	Mammalian
Vam3p	EGFR
Fth1p	GHR
Mammalian	IL-2ß
LAMP-1	B-AR
LAMP-2	CXCR4
HLA-DM	**Plasma membrane**
	Transmembrane proteins
	Yeast
	Fur4p
	Ino1p
	Mal61p
	Gap1p
	Tat2p
	Pdr5p
	Ste6p
	Mammalian
	E-Cadherin
	EnaC
	MHC II
	CD 63
	CD 81
	CD 82
	Biosynthetic
	Transmembrane proteins
	Yeast
	Cps1p
	Phm5p
	Sna3p
	Mammalian
	LAP
	CI-M6PR

Trafficking between Golgi and MVB

In mammalian cells, membrane trafficking routes of transmembrane proteins from TGN to endosomes have been described. Many membrane proteins and lumenal hydrolases in the TGN destined for MVBs are sorted and enriched into vesicles by three independent adaptor/coat protein complexes. GGAs (Golgi-localizing, gamma-adaptin ear homology domain, ARF-binding) are ATP ribosylation factor (ARF) dependent coats involved in sorting of membrane proteins such as vacuolar ATPase, M6PR and luminal proteins such as cathepsins into vesicles at TGN. AP-1 adaptor complex composed of large subunits γ-adaptin, β1-adaptin and small subunits μ1-adaptin, σ1-adaptin defines another pathway of trafficking between the Golgi and MVB (Reusch et al. 2002). The third pathway of membrane trafficking between Golgi and endolysosomes is dependent on AP-3 adaptor complex. AP-3 adaptor complex is similar to AP-1 adaptor complex in its composition except for the large δ-adaptin subunit that differs from AP-1 γ-adaptin subunit. AP-3-adaptor protein complex recognizes sorting signals that are either an acidic residue upstream of di-leucine or a tyrosine present in cytoplasmic domain of membrane proteins such as LAMP-1 and LIMP-2 and recruits clathrin (Dell'Angelica et al. 1999, Faundez et al. 1998). AP3 has been shown to be involved in endosomal sequestration and targetting of LAMP-1 and LAMP-2 to lysosomes from tubular sorting endosomes (Peden et al. 2004). AP-3 has been shown to have clathrin-independent function as well (Robinson 2004). The molecular players that regulate fusion of Golgi derived vesicles with MVBs in mammalian cells are yet unknown. In fact, controversy reins in terms of whether delivery of proteins from the TGN to the endolysosome takes place via the early endosomal system or to the late endosome lysosome directly, or if there are multiple points of intersection between these two membrane systems (Pfeffer 2003).

Biogenesis of MVB: lessons from yeast mutants

Genetic studies investigating yeast vacuole biogenesis have provided insights about molecules that are likely to regulate these processes (Stack et al. 1995). It has been observed that many proteins involved in vacuolar sorting and biogenesis in yeast have orthologues in higher eukaryotes such as *Drosophila* and mammals where they are likely to regulate biogenesis of late endosomes and lysosomes (Mullins and Bonifacino 2001). Genetic screens scoring for mutations in the delivery of a CPY-invertase fusion protein that is normally delivered to

vacuole have isolated *vacuolar protein sorting* (vps) mutants that collectively define 40 complementation groups (Banta et al. 1988, Robinson et al. 1988). Vps gene products constitute components of a molecular apparatus responsible for recognition, packaging and vesicular transport of proteins to yeast vacuole. Based on vacuole morphology in mutants, Vps genes are classified as class A-F. These genes regulate specific steps in vacuolar membrane trafficking as shown in figure 3.

In addition to these genes, there are a host of adaptors and coat-proteins identified in these and other assays that act to direct and regulate membrane traffic to and from the yeast vacolar system. Membrane proteins such as carboxypeptidase S (CPS) or Vps10p and lumenal carboxypeptidase Y (CPY) are sorted at TGN by GGA. AP-3 directs the vacuolar membrane proteins alkaline phosphatase (ALP) and Vam3p directly to the vacuole via a pathway different from AP-1 dependent pathway and GGA regulated CPY pathway (Odorizzi et al. 1998b). AP-3 dependent sorting of membrane proteins to the vacuole is clathrin-independent (Vowels and Payne, 1998) but is likely to be coat-protein Vps41p dependent (Rehling et al. 1999).

Class D mutants have normal to enlarged vacuoles and are defective in delivery of vacuolar ATPase and CPY to vacuoles. Class D Vps proteins are likely to play a role in delivery of Golgi derived biosynthetic cargo to PVE. PI3 Kinase Vps34p synthesizes PI3P on endosomes that recruits Vac1p, a multivalent adaptor protein to the compartment. Vac1p is involved in docking and fusion of Golgi-derived vesicles with pre-vacuolar endosome (PVE) by integrating both phosphoinositide (Vps34p) and GTPase (Vps21p) signals essential for Vps45p-Pep12p function (Peterson et al. 1999). Vps45p is Sec1p homologue that binds soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) Tlg2p and Pep12p on endosomal membranes (Nichols et al. 1998). Interaction between Sec1 family of proteins and SNAREs is a prerequisite for endosomal membrane fusion.

Class E Vps mutants accumulate a novel organelle distinct from the vacuole that is likely to represent an exaggerated PVE similar to that identified in wild-type cells using biochemical techniques (Vida et al. 1993). The PVE shows differential localization of membrane proteins in lumenal vesicles and limiting endosomal membrane (Table 2). 17 class E Vps proteins implicated in sorting of proteins into MVB have been listed (Table 3), functional loss of any one leads to malformed late endosomes referred as class E compartment.

Sorting of proteins in MVBs

The functional significance and identity of the endolysosomal system, and consequently MVBs is defined by a unique set of proteins that reside within

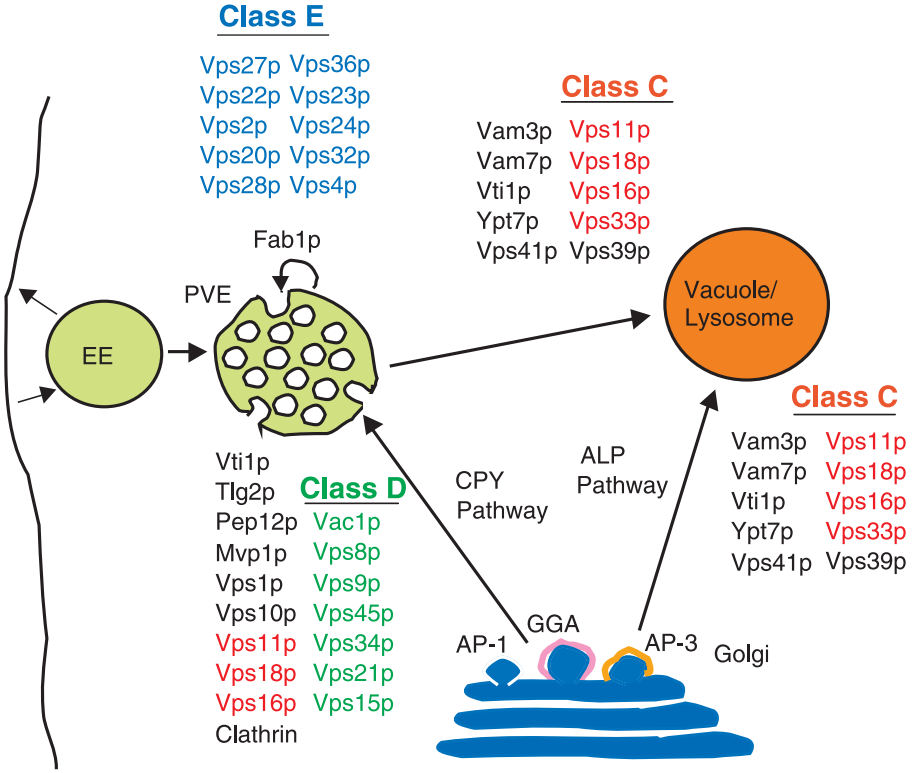

Figure 3: *athways of membrane trafficking between TGN and vacuole in yeast*
Alkaline phosphatase (ALP) containing vesicles directly fuse with vacuole while carboxypeptidase Y (CPY) is delivered to pre-vacuolar endosome prior to vacuole. The molecular players that regulate vesicle budding, transport and fusion with target membranes in these pathways have been listed. Class D (green), class E (blue) and class C (red) Vps proteins have been indicated

this organelle (Table 2). Therefore, the accurate and efficient delivery of these proteins *to* as well as *into* the MVB is of fundamental importance in establishing and maintaining late endosomal structure and function. Plasma membrane proteins and fluid are internalized via endocytic pathway and delivered to late endosomes. Biosynthetic cargo containing vesicles from Golgi deliver cargo to MVBs. Some of the membrane proteins that are delivered to MVB are subsequently sorted into lumenal vesicles while others are retained on the limiting membrane or recycled back to Golgi (Piper and Luzio, 2001). Membrane proteins that are sequestered into lumenal vesicles of MVB have different fates. Cell surface receptors such as epidermal growth factor receptor (EGFR) are degraded, proteins such as CD63, CD81 and CD82 remain stable in lumenal vesicles while M6PR is recycled back to TGN (Piper and Luzio 2001).

Recent studies have shown that mono-ubiquitination can serve as a signal for sorting of transmembrane proteins into the lumenal vesicles of MVB (Hicke 2001, Marchese and Benovic 2001, Rocca et al. 2001). Recombinant fusion of a recycling receptor such as transferrin receptor with ubiquitin results in its lysosomal targeting (Raiborg et al. 2003, Urbanowski and Piper 2001). Ubiquitin, a conserved 76 amino-acid (aa) polypeptide is linked covalently to protein substrates through the activity of a cascade of enzymes (Katzmann et al. 2002). Ubiquitin ligases such as Tul1 in yeast, Neuralized in *Drosophila* or *Xenopus*, Mind-bomb in zebrafish and cbl in mammalian cells are involved in ubiquitinating Golgi or cell surface proteins resulting in their targeting into MVB (Deblandre et al. 2001, Itoh et al. 2003, Lai et al. 2001, Pavlopoulos et al. 2001, Reggiori and Pelham 2002). The ubiquitin tag on the protein is recognized by ubiquitin-interacting motif (UIM) or ubiquitin E2 variant (UEV) domain present in putative cytosolic regulators of MVB sorting such as Vps27p and Vps23p in yeast (Bilodeau et al. 2002, Shih et al. 2002) or their homolgues Hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) and Tsg101 in mammalian cells (Aguilar et al. 2003, Lloyd et al. 2002, Raiborg et al. 2001, Raiborg et al. 2003).

Hrs binds enriched regions of PI3P on the limiting membrane of endosomes via its FYVE domain (Raiborg et al. 2001). The C-terminus of Hrs contains a functional clathrin box motif that interacts directly with the terminal beta-propeller domain of clathrin heavy chain recruits flat, bilayered clathrin coats on endosomes (Raiborg et al. 2001, Sachse et al. 2002). Bilayered clathrin coat on endosomes is thought to enrich ubiquitinated transmembrane proteins destined for lumenal vesicles of the MVB via their interaction with Hrs (Raiborg et al. 2001, Sachse et al. 2002). The mechanism involved in the inward budding of the endosomal membrane is poorly understood. A candidate cytosolic protein ALIX functions as a negative regulator of multi-vesicularization. ALIX binds endosomal LBPA rendering it unavailable for its role in inward budding of endosomal membrane (Matsuo et al. 2004).

A similar paradigm of sorting membrane proteins into the vacuole lumen exists in yeast. Vps27p and Vps23p sort endocytosed α-factor receptor, biosynthetic CPS and ubiquitin fusion reporter construct into the vacuole lumen (Bilodeau et al. 2002, Shih et al. 2002). Although genetic evidence in yeast suggests that *Vps27* acts upstream of *Vps23* (Katzmann et al. 2003), the functional relationship between these proteins is unclear. Vps27p and Vps23p are amongst 17 class E Vps proteins (Table 3) involved in sorting of MVB proteins that have been identified; functional loss of any one leads to malformed late endosomes referred as class E compartment (Piper and Luzio, 2001). Vps27p delivers

Table 2:

Membrane proteins	Lumenal proteins
Acetyltransferase	Acid phosphatase
Acid phosphatase	Arylsulfatase B
β-Glucosidase	Cathepsin B-I
LAMP-1	Cathepsin B-II
LAMP-2 (MAC-3)	Cathepsin D
Lgp 80	Cathepsin L
Lgp 100	α-Galactosidase
Lgp 120	β-Galactosidase
LIMP-2	α-Glucosidase
LIMP-3	β-Glucuronidase
CV-24 antigen	β-Hexosamindase A
Carboxypeptidase S	β-Hexosamindase B
	Phospholipase A
	Carboxypeptidase Y
	Carboxypeptidase S
	Proteinase A
	Proteinase B
	α-Mannosidase

Table 3:

Class E Vps proteins	Protein complex
Vps23p	ESCRT-I
Vps28p	ESCRT-I
Vps37p	ESCRT-I
Vps22p	ESCRT-II
Vps25p	ESCRT-II
Vps36p	ESCRT-II
Vps2p	ESCRT-III
Vps20p	ESCRT-III
Vps24p	ESCRT-III
Vps32p/Snf7p	ESCRT-III
Vps27p	With Hse1p
Hse1p	With Vps27p
Vps4p	Oligomer
Did2p	Not known
Vps60p	
Vps31p	
Vps44p	

the ubiquitinated membrane protein to ESCRT-I (endosomal sorting complex required for transport-I), a protein complex that includes Vps23p, Vps28p and Vps37p (Katzmann et al. 2003). ESCRT-I, ESCRT-II and ESCRT-III are class E protein complexes that are involved sequentially in protein sorting into MVB [Table 3, Fig. 2, (Raiborg et al. 2003)]. ESCRT- I functions upstream of ESCRT-II on the endosomal membrane and relays the membrane protein (Babst et al. 2002) to ESCRT-II, a 155 KDa complex localized transiently to the membrane which recruits ESCRT-III. ESCRT-III is composed of two functional sub-complexes and is stabilized on the membrane by myristoylation of Vps20p (Babst et al. 2002) . The membrane proximal sub-complex Snf7p-Vps20p is required for the peripheral association of Vps2p-Vps24p sub-complex. The peripheral Vps2p-Vps24p protein complex recruits a de-ubiquitylating enzyme Doa4p and an AAA type ATPase Vps4p. De-ubiquitylation of the membrane protein precedes its localization into the lumenal vesicles of MVB. Vps4p is involved in catalyzing dissociation of all ESCRT complexes from the endosomal membrane prior to delivery of the ubiquitinated receptor into the lumenal vesicles (Babst et al. 1998).

Proteins of mammalian ESCRT are beginning to be identified (Babst et al. 2000, Stuchell et al. 2004, von Schwedler et al. 2003). In MDCK cells, a dominant negative form of mammalian homologue of yeast *Vps4* disrupts EGFR degradation and results in accumulation of aberrant class E such as compartments (Fujita et al. 2003) suggesting a conserved role for ESCRT proteins in sorting membrane proteins into MVB in higher metazoa.

Although mono-ubiquitination has been shown to be an important sorting determinant for targeting proteins into MVBs, non-ubiquitinated transmembrane proteins are also sorted into MVBs. In yeast, reduction in levels of unconjugated ubiquitin does not affect targeting of membrane protein Sna3p into MVB (Reggiori and Pelham 2001). The protein machinery involved in recognition and sorting of non-ubiquitinated proteins is very poorly understood. An interesting candidate protein is cytosolic GASP (G-protein-coupled receptor-associated sorting protein) that binds to non-ubiquitinated δ-opioid receptors and targets them into MVBs (Whistler et al. 2002) .

Unanswered issues in MVB biogenesis

Questions regarding mechanism of formation of lumenal vesicles of MVB, their fission from the limiting membrane, re-fusion with limiting membrane and possible role of lipids in sorting of non-ubiquitinated proteins remain unaddressed.

Multi-lamellar late endosomes

Late endosomes also exhibit multi-lamellar morphology as observed using EM (Kobayashi et al. 1998). Multi-lamellar late endosomes (MLE) are characterized by whorl of membranes within the limiting endosomal membrane that may in many instances include lumenal vesicles (Reaves et al. 2000). Similar to MVBs, the lumenal membranes of MLE are enriched for LBPA (Kobayashi et al. 1998). Experiments done on mammalian cells and *Drosophila* suggest that MLEs could form from MVBs in the endocytic itinerary. It has been observed that BSA-gold is endocytosed into MVBs prior to MLE when human B lymphocytes are incubated with BSA-gold for 10 min followed by incubation in absence of the probe (Mobius et al. 2003). In *Drosophila* R7 photoreceptor cells, HRP tagged bride of sevenless, a ligand for sevenless receptor is endocytosed into MVBs and subsequently localizes to MLE (Sunio et al. 1999). The mechanism involved in formation of MLEs from MVBs is poorly understood. It has been observed that cholesterol is efficiently removed from MVBs while enriching for lysosomal proteins such as LAMP during MLE formation (Mobius et al. 2003).

MLEs seem to be induced during autophagy (Hariri et al. 2000) and are capable of fusion with autophagosomes (Lucocq and Walker 1997). In antigen presenting cells such as macrophages, MLEs have been shown to contain peptide-MHC complexes essential for antigen presentation (Geuze 1998). In yeast, MLEs are observed only in class E mutants that affect vacuole biogenesis (Rieder et al. 1996). As yet, fundamental molecular differences between MLEs and MVBs remain to be established; their distinctness appears to rest upon a subtle morphological difference.

Lysosomes: organization, composition and biogenesis

Lysosomes represent the next stage in the biogenesis of the endolysosomal system; cargo internalized from the cell surface appears in this set of organelles right after traversing late endosomes. Lysosomes are extremely dynamic organelles undergoing large-scale morphological transformations that show tubular (Racoosin and Swanson 1993, Sriram et al. 2003), spherical and electron dense (Bright et al. 1997) or multi-lamellar morphology (Sunio et al. 1999) depending on the cell type they have been visualized in (or procedures used to fix cells). Their morphology and dynamics depend on the structural role of actin filaments and microtubules. Lysosomes display a combination of microtubule dependent rapid long-range directional movements and actin dependent short random movements and pauses (Cordonnier et al. 2001). In addition, microtubules are required for maintenance of

lysosome distribution in its juxtanuclear position and facilitate delivery of ligands to the degradative compartment. Actin filaments and myosin I-alpha contribute to the movement of lysosomes in cooperation with microtubules and their associated molecular motors (Cordonnier et al. 2001, Durrbach et al. 1996).

Composition of lysosomes

Lysosomal membrane is rich in glycoproteins and distinguished from late endosomes by the absence of M6PR (Mullins and Bonifacino 2001, Piper and Luzio 2001). These glycoproteins are thought to form a glycocalyx matrix in the lysosome thus protecting the lysosomal membrane from lumenal proteases and lipases. The integral membrane proteins and lumenal enzymes found in lysosomes and lysosome-like yeast vacuole have been listed in table 2. In addition to glycoproteins such as NPC 1 that regulates cholesterol levels in lysosomes (Neufeld et al. 1999), lysosomal membrane is rich in transporters for amino acids (Schneider et al. 1984), fatty acids (Rome et al. 1983), carbohydrates (Cohn and Ehrenreich, 1969, Maguire et al. 1983, Renlund et al. 1986) and nutrients [such as cobalamin (Vitamin B_{12}), (Rosenblatt et al. 1985)] that are generated as products of degradation.

The lumen is rich in lipofuscins, cysteine (Cathepsin B, L, S and H) and aspartate (Cathepsin D, E) proteases, lipases and acid hydrolases. Lipofusins are polymeric substances composed of cross-linked protein residues that are not degraded in lysosomes (Brunk and Terman, 2002). Cathepsins, important constituents of lysosomal lytic system are delivered to late endosomes via M6PR in non functional pro-form that undergoes processing in acidic lysosomes to result in mature functional enzyme. Some soluble lumenal proteins such as mature human lysosomal acid phosphatase are synthesized as integral membrane protein precursors (Pohlmann et al. 1988, Waheed et al. 1988) and targeted to lysosomes where they are proteolytically cleaved into mature enzymes. Most of soluble lysosomal proteins have a half-life of 24 h (Mort and Buttle, 1997).

Biogenesis of lysosomes: membrane trafficking from MVBs to lysosomes/vacuole

Once again studies in yeast have yielded information regarding this step in membrane trafficking in the endo-lysosomal system. The fusion of the late endosome equivalent, the pre-vacuolar endosome (PVE) with yeast lysosome equivalent, the vacuole, requires class C Vps proteins Vps18p, Vps11p, Vps16p, Vps33p, another protein complex containing Vps41p and Vps39p, GTPase Ypt7p and SNAREs- Vti1p, Vam7p, Vam3p [Fig 4, (Nakamura et al. 1997, Rieder and Emr 1997)]. Class C Vps proteins are coiled-coil containing proteins that form hetero-oligomeric protein complexes that localize to vacuolar membrane. *Vps18*^{ts} mutant at the non-permissive temperature is defective in processing of both CPY and ALP into their mature forms and degradation of endocytosed Ste6p transporter. It accumulates MVBs, vesicle transport intermediates and auto-phagosomes, a phenotype rescued by over-expression of Vps16p or SNARE Vam3p (Rieder and Emr, 1997). Vps33p ATPase belongs to Sec1 family of SNARE interacting proteins (Gerhardt et al. 1998). Vps11p binds to the COOH-terminal of Vps39p of Vps41p-Vps39p protein complex thus organizing HOPS (homotypic fusion and vacuole protein sorting) complex (Ungermann et al. 2000, Wurmser et al. 2000). Vps39p-Vps41p complex functions as a downstream effector as well as nucleotide exchange factor of active, GTP-bound form of Ypt7, a Rab GTPase required for tethering transport vesicles to vacuole (Price et al. 2000, Wurmser et al. 2000). A possible mechanism by which membrane fusion is brought about by class C Vps proteins as part of HOPS protein complex is schematically represented in Figure 4. Vps41p-Vps39p protein complex is required not only for CPY pathway to vacuole via PVE but also in ALP pathway [(Stepp et al. 1997), Fig 3], ALP containing vesicles from TGN bypass PVE and fuse directly with vacuole (Fig 3).

Class C Vps genes might also function at an earlier step in the formation of PVE in Golgi-vacuole membrane trafficking as shown by their genetic interaction with class D Vps genes. Vps11p binds Vac1p in vitro and Vps18p binds Vac1p in a two-hybrid assay suggesting a probable role for class C Vps complex in early stages of docking of TGN derived transport vesicles with PVE [Fig 3, (Peterson and Emr, 2001)]. The role of Sec1p homologue Vps33p in the biogenesis of PVE remains to be demonstrated.

In stark contrast to the studies in yeast, experiments done using *Drosophila* seem to suggest that homologues of class C Vps proteins differentially regulate distinct steps in lysosomal membrane trafficking in higher metazoa (Sriram et al. 2003). *Drosophila* eye color genes *deep-orange* (*dor*), *carnation* (*car*) and *light* (lt) are homologues of yeast *Vps18*, *Vps33* and *Vps41* respectively (Sevrioukov et al. 1999, Shestopal et al. 1997, Warner et al. 1998) that interact with each other genetically (Lindsley and Zimm 1992). It has been demonstrated *in vitro* that Dor and Car can form a multi-protein complex (Sevrioukov et al. 1999). However, immuno-fluorescence microscopy has revealed that while Dor and Car associate with Rab7 positive, Hrs positive MVBs (Sriram et al. 2003), Car regulates Dor function on MVBs. The MVBs undergo a morphological transformation to form smaller Rab7 positive pre-lysosomal compartments that are Hrs negative (Sriram et al. 2003). These pre-lysosomal compartments

subsequently fuse with tubular lysosomes (Sriram et al. 2003). Unlike its yeast homologue that localizes to vacuole, Dor is not present on pre-lysosomal and lysosomal compartments. Car on the other hand labels Rab7 positive, Hrs and Dor negative pre-lysosomal compartments and regulates their fusion with lysosomes (Sriram et al. 2003).

A clone of cells in *Drosophila* compound eye lacking Dor shows increased steady state levels of Bride of sevenless, a sevenless receptor ligand suggesting a defect in endosomal degradation of ligand-receptor complex (Sevrioukov et al. 1999). Hemocytes from *dor*^{1}mutant larvae show defect in degradation of endogenous scavenger receptors. This defect in degradation of endocytosed receptors is caused by lack of fusion of Golgi derived vesicles rich in proteases with MVBs. Thus Dor regulates fusion of Golgi derived vesicles with MVB (Sriram et al. 2003). The SNAREs regulating these membrane fusion steps are beginning to be described (see section 4).

In animal cells, mammalian homologues of yeast Ypt7p, class C Vps proteins and SNAREs likely to be involved in lysosome biogenesis have been identified (Bucci et al. 2000, Caplan et al. 2001, Kim et al. 2001, Mullock et al. 2000, Nakamura et al. 2000, Poupon et al. 2003). Expression of dominant negative Rab7 mutants, GFP-Rab7T22N and GFP-Rab7N125I causes dispersal of LAMP-1, cathepsin D positive lysosomes without affecting M6PR (late endosomal marker) localization. This also results in lysosomes becoming inaccessible to endocytosed low-density lipoprotein. Thus Rab7 is involved in delivery of endocytosed cargo from late endosomes to lysosomes in addition to maintenance of perinuclear lysosomes in the cell (Bucci et al. 2000).

Mammalian class C Vps proteins localize to LAMP-1, Lgp positive compartments and associate with mVps39p and syntaxin-7 (Vam3p SNARE homologue) as revealed by immuno-precipitation and gel filtration studies (Kim et al. 2001, Poupon et al. 2003). siRNA-induced decrease in cellular mVps18p results in dispersal of Lgp positive compartments away from their juxtanuclear location in the cell (Poupon et al. 2003). Increasing levels of mVps39p-GFP in this background results in perinuclear clustering of Lgp positive compartments arguing for mVps39p function either downstream or parallel to mVps18p function. Increasing cellular levels of mVps18p-GFP or mVps39p-GFP results in lysosome clustering in juxtanuclear region (Bucci et al. 2000, Caplan et al. 2001, Poupon et al. 2003). Unfortunately, the lack of discrimination between late endosomes and lysosomes in these studies has left questions about compartment specificity of mVps39p and mVps18p unanswered.

These data suggest that while many molecular players involved in biogenesis of lysosomes are conserved across different model systems the role of these molecules in higher metazoa as compared to yeast is likely to be system-specific, since many of the steps involved in lysosomal membrane trafficking are differentially organized.

SNARE-mediated membrane fusion in the endo-lysosomal system

The SNARE hypothesis

One of the main insights into understanding factors that govern membrane fusion inevitable in communicating between distinct membrane compartments inside the cell has been the discovery of the rules behind SNARE pairing (McNew et al. 2000). The SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) family of proteins constitutes the basic protein machinery required for all intracellular membrane fusion events (Chen and Scheller 2001). They are anchored to the membrane by a C-terminal transmembrane domain or by acyl-derivatization and contain conserved heptad repeat sequences in their membrane proximal regions that form coiled-coil structures (Chen and Scheller 2001). SNARE complexes exist in *cis* form (SNAREs anchored to the same membrane interacting laterally) or in *trans* form (SNAREs anchored on the opposed membranes of docked organelles interacting in *trans*). They form large 20S complex with the aid of a molecular chaparone, the AAA type ATPase NSF (*N*-ethylmaleimide-sensitive fusion protein; yeast Sec18p) and SNAPs (soluble NSF attachment proteins; yeast Sec17p).

SNAREs are classified as R-SNAREs and Q-SNAREs that contain arginine and glutamine respectively as a highly conserved residue (Fasshauer et al. 1998). They are also categorized as members of syntaxin, VAMP (vesicle-associated membrane protein) or SNAP-25 (25kDa synaptosome-associated protein) families. Their intrinsic physio-chemical properties in combination with upstream targeting and tethering factors such as Rab proteins and Rab effectors are thought to encode aspects of specificity in intracellular membrane transport (Chen and Scheller 2001).

SNARES in endolysosomal membrane traffic

Biochemical analyses of yeast homotypic vacuolar fusion have provided a tentative functional sequence of tethering and targeting proteins involved in SNARE mediated vacuolar membrane fusion (Wickner 2002). Based on these data, vacuolar membrane fusion event can be dissected into three steps (Fig. 4).

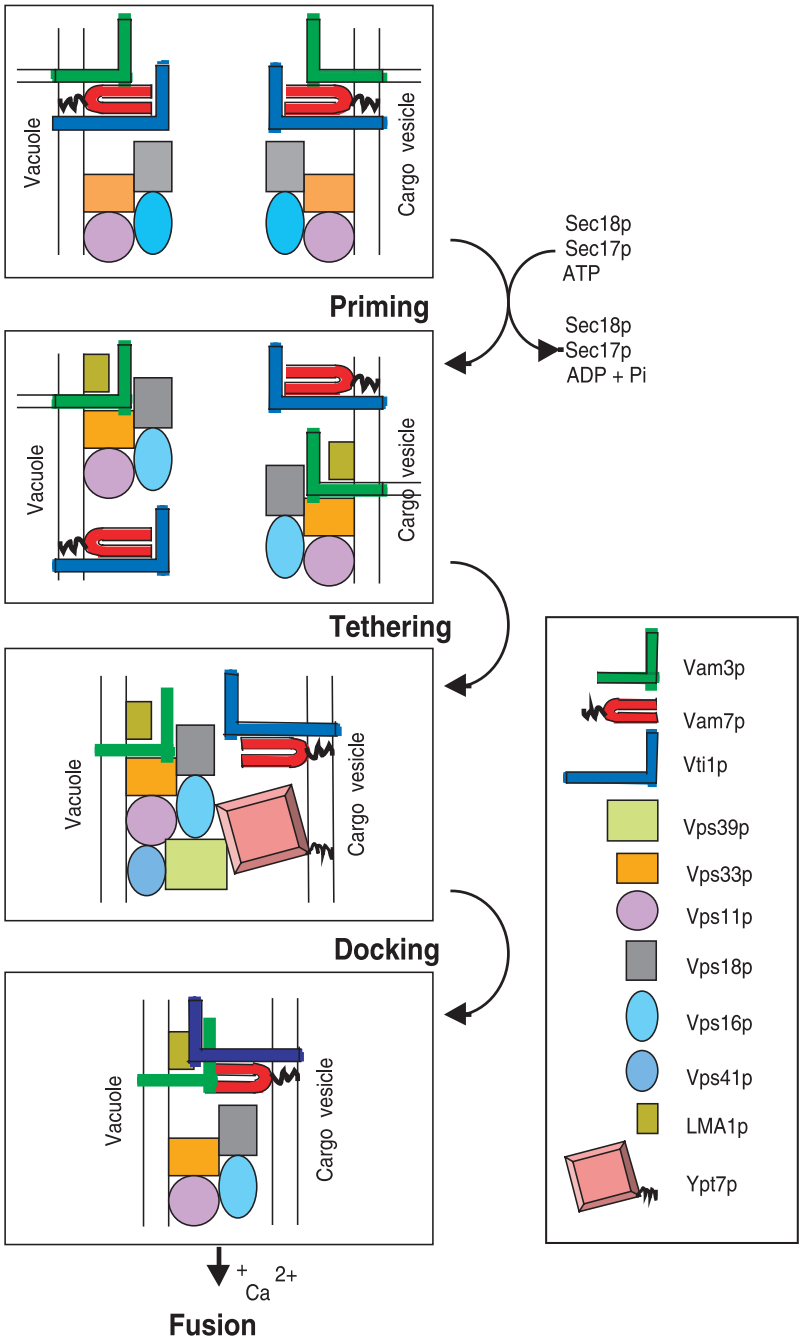

Figure 4: *Schematic showing steps suggested to be involved in class C Vps protein complex regulated SNARE mediated membrane fusion between pre-vacuolar endosome (PVE) and vacuole or cargo vesicles and vacuole. During priming, Sec18p ATPase hydrolyses ATP and releases Sec17p off cis-SNARE complex on both the membranes. Vps33p (class C Vps protein) interacts with Vam3p driving the reaction towards docking. Vps11p of class C Vps protein complex interacts with Vps39p-Vps41p. Vps39p-Vps41p interaction with class C Vps protein complex constitutes HOPS complex that functions as Ypt7p guanine nucleotide exchange factor as well as an Ypt7p effector. The Ypt7p effector complex is involved in tethering the two membranes. Docking results from* trans*-SNARE pairing that involves interaction between SNAREs on opposing membranes and release of class C Vps protein complex from Vam3p. Vam3p, Vti1p and Vam7p in* trans*-SNARE interaction cause* trans *interaction of V_0 subunits of opposing membranes prior to membrane fusion and mixing of lumenal contents between PVE or cargo vesicle and vacuole.*

1) Priming is ATP-driven modification of the association status of SNAREs, Rabs and effector complexes.
2) Docking is initiated by Rab and its effectors leading to trans-SNARE pairing.
3) Membrane fusion is initiated by calcium flux resulting in fusion of membrane bilayers and mixing of lumenal contents.

Priming involves hydrolysis of ATP by Sec18p resulting in Sec17p release from interaction with SNAREs on both fusion membranes. This results in disassembly of the *cis*-SNARE complex on both fusion membranes that can be guided into a productive fusion event only if docking occurs rapidly. Priming requires phosphatidylinositol 4,5 bisphosphate [PI(4,5)P_2, (Mayer

et al. 1996)], ergosterol (Kato and Wickner 2001) and LMA1, a co-chaperone that is initially bound to Sec18p but subsequently associates with SNARE Vam3p (Xu et al. 1997, Xu et al. 1998). It is also observed that HOPS complex activates Ypt7p (Wurmser et al. 2000) and remains bound as Ypt7p effector (Seals et al. 2000). Class C Vps complex maintains Vam3p (SNARE) in an unpaired state (Wurmser et al. 2000), a step critical for guiding the reaction into a productive fusion event. This interaction is likely to be between Sec1p homologue Vps33p and Vam3p. PI3P (Cheever et al. 2001) and activated Ypt7p (Ungermann et al. 2000) are required for maintenance of SNARE Vam7p (SNAP-25 homologue) on the vacuole. The mechanism of loading and maintenance of HOPS on vacuolar membrane remains poorly understood.

Docking refers to the sequence of events that occurs upon contact between the vacuoles, establishing their stable interaction, thus committing them to subsequent fusion. Ypt7p and HOPS complex trigger two docking events that are activation of Rho1p and Cdc42p (Eitzen et al. 2001, Muller et al. 2001) and formation of trans-SNARE pairs (Ungermann et al. 1998). During this process, class C Vps protein complex releases Vam3p, resulting in trans-SNARE complexes containing Vti1p, Vam3p, and Vam7p. Extent of vacuole acidification (Ungermann et al. 1999) and membrane PI(4,5)P_2 (Mayer et al. 2000) influences docking. Docking results in release of calcium from the vacuole (Peters and Mayer 1998) which activates calmodulin to bind to V_0, the integral domain of vacuolar H^+-ATPase. This triggers a *trans*-complex formation between V_0 complexes on opposed vacuoles (Peters et al. 2001) that also contains Vam3p. Protein phosphatase 1 releases LMA1 from the vacuoles prior to membrane fusion and contents mixing (Conradt et al. 1994, Peters et al. 1999, Xu et al. 1998).

SNARE pairing regulates homotypic versus heterotypic fusion of late endosomes and lysosomes

Syntaxin-7 (Q-SNARE) regulates late endosomal and lysosomal membrane fusion; microinjection into cells of anti-syntaxin-7 antibodies or bacterially expressed syntaxin-7 lacking the transmembrane domain inhibits fusion of late endosomes with lysosomes and lysosomes with lysosomes without any effect on early endosomal fusion (Ward et al. 2000). Interestingly, the SNARE complex that provides specificity for heterotypic fusions between late endosomes and lysosomes (Pryor et al. 2004)has been shown to be different from the SNARE complex mediating homotypic fusions between late endosomes [(Antonin et al. 2002, Antonin et al. 2000)]. The trans-SNARE complex consisting of Q-SNAREs syntaxin 7, Vti1b and syntaxin 8 and the R-SNARE, VAMP8, are responsible for homotypic fusion of late endosomes in a cell free system [(Antonin et al. 2002, Antonin et al. 2000)]. At the same time the same Q-SNAREs can combine with an alternative R-SNARE, namely, VAMP7, for heterotypic fusion between late endosomes and lysosomes using antibody inhibition experiments in rat liver cell free system (Pryor et al. 2004), suggesting that the regulation of Q-SNARE partners can dictate homo- versus hetero-typic fusion in the endolysosomal system. Experiments using siRNA or antibody microinjections targeting specific candidate molecules followed by visualizing membrane trafficking in live cells is likely to provide further insights into regulatory mechanisms of molecular players in lysosome biogenesis.

Trafficking paradigms and organelle identity in late endo-lysosomal biogenesis

Early to late endosome traffic

Membrane trafficking between early endosomes to late endosomes has been explained chiefly by two main trafficking paradigms. These are "maturation model" and "stable compartment model or vesicle shuttle model" [(Gruenberg and Maxfield, 1995)] are depicted in Fig. 5. In the maturation model, early endosomes form *de novo* by coalescence of vesicles from the plasma membrane; the composition of early endosomes then changes due to removal of recycling cargo and addition of TGN- derived vesicles, till they eventually 'mature' into late endosomes and then lysosomes. The vesicle-shuttle model postulates that early endosomes, late endosomes and lysosomes are stable compartments in the cell. Transport proceeds from early endosomes through endosomal carrier vesicles (ECV) to preexisting late endosomes with characteristics of MVBs or directly to lysosomes.

Each model makes different predictions about the nature of trafficking of proteins that are responsible for endosome sorting and transport. The tight regulation of membrane trafficking requires that specific tethering and targeting factors are present on each endosomal compartment membrane to enable the docking of specific transport vesicles. According to maturation model, these tethering and targeting factors must first be inactivated and then either removed by recycling or degraded in lysosomes during the maturation process. In contrast, the stable compartment model predicts that these factors may remain as resident components of the stable endosomal membrane.

Experimental evidence supporting the two models of membrane traffic in the endolysosomal system

Experiments done on HepG2 cells looking at endocytosis of ^{125}I- or HRP-labeled asialoorosomucoid receptor (ASOR) or transferrin receptor (TfR) by Stoorvogel and

co-workers suggested that late endosomes are derived from early endosomes by maturation (Stoorvogel et al. 1991). It was observed that Tf-HRP was internalized into endosomes that showed a gradual decrease in TfR levels post formation and a concomitant increase in M6PR contents. The entire endocytic pathway of ^{125}I-ASOR (a protein that was delivered to late endosomes and lysosomes) remained accessible to newly endocytosed Tf-HRP until a compartment with a density near to that of lysosomes was reached. Endocytosed Tf-HRP was also localized in M6PR containing endosomes. It was also observed that M6PR also labeled early endosomes indicating the absence of two separate stable pools of endosomes, early and late. These data are consistent with maturation model of membrane trafficking.

Experiments done on CHO cells using fluorescently labeled LDL by Dunn KW and Maxfield FR (1992) also demonstrated that delivery of ligands from early endosomes to late endosomes occurred by maturation process (Dunn and Maxfield, 1992). In this study, CHO cells were incubated with diO-LDL, followed after a variable chase, by diI-LDL and the amount of diO in diI containing endosomes measured. As the chase period was lengthened, an increasing fraction of endosomes containing diO-LDL from the initial incubation had no detectable diI-LDL from the second incubation. Interestingly, those endosomes that contained both probes showed no decrease in amount of diO-LDL per endosome. These data are consistent with a maturation mechanism in which early endosome retains and accumulates lysosomally directed ligands until it loses its ability to fuse with newly formed endocytic vesicles and matures into a late endosome. At the same time the early endosome is continuously recycling membrane components to the cell surface, providing an explanation for the efficient recycling of non-specific membrane components from early endosomes (Dunn and Maxfield 1992, Mayor et al. 1993). This recycling activity is likely to gradually come to an end after maturation (Stoorvogel et al. 1991).

Experiments on mouse macrophages by Racoosin EL and Swanson JA (1993) showed that macropinosomes matured by progressive acquisition and loss of characteristic endocytic vesicle markers (Racoosin and Swanson, 1993). Macropinosomes were labeled for 1 min with fluid phase probe, fluorescein dextran, chased for various times and change in their antigenic profile monitored. It was observed that some newly formed macropinosomes were positive for TfR, but few were Rab7 or Lgp-A positive. After a chase of 2-4 min, >60% of macropinosomes stained for Rab7 and Lgp-A and were negative for TfR. After 9-12 min chase, most macropinosomes were Lgp-A positive while only a few were Rab7 positive. These results indicated that after formation, macropinosomes acquired, and then lost specific membrane markers for organelles in the endocytic pathway.

Experiments with primary hemocyte cultures from *Drosophila* larvae, using fluorescently labeled dextran (Fl-Dex) and maleilated bovine serum albumin (Fl-mBSA) by Sriram et.al. (2003) demonstrated that delivery of ligands from multi-vesicular Rab7 positive endosomes to prelysosomal compartments occurred by maturation process (Sriram et al. 2003). Primary hemocyte cultures from wild-type larvae were incubated with Fl-Dex, followed after a variable chase, by Fl-mBSA and the amount of Fl-Dex in Fl-mBSA containing endosomes measured. As the chase period was lengthened, an increasing fraction of endosomes containing Fl-Dex from the initial incubation had no detectable Fl-mBSA from the second incubation. Interestingly, those endosomes that contained both probes showed no decrease in amount of Fl-Dex per endosome. These data were consistent with a maturation mechanism in which MVBs retain and accumulate lysosomally directed ligands until they lose ability to fuse with newly formed MVBs and mature into prelysosomal compartments.

Arguments in favour of a stable compartment model come from experiments carried out in BHK cells where putative endocytic carrier vesicles between early endosomes and late endosomes were identified (Gruenberg et al. 1989). In these experiments, the transmembrane glycoprotein VSVG of vesicular stomatitis virus was internalized for different times and immuno-stained using antibodies generated against the cytoplasmic domain of the VSVG protein. EM visualization showed that post their appearance in early endosomes, the molecules targeted for degradation were observed in distinct spherical vesicles (0.5 μm) that were negative for acid phosphatase, markers for late endosomes and lysosomes. Microtubule depolymerization resulted in internalized molecules remaining in spherical vesicles and without being delivered to acid phosphatase positive compartments. Interestingly, post microtubule depolymerization, the spherical compartments were not accessible to early endosomal elements suggesting that they were transport intermediates (vesicle-shuttle model) from early endosomes *en route* to acid phosphatase positive late endosomes and lysosomes.

A potential resolution of the stable vs maturation model controversy

Like a lot of biological processes, adherence to either of the two models cannot provide an explanation for all the disparate observations of the transformation of early endosomal identity, and the presence of 'carrier vesicles'

simultaneously. However, a model that combines aspects of the two modes of transport provides an interesting explanation of *all* the seemingly disparate observations. Consider that early endosomes do mature gradually; retaining lysosomally destined cargo and recycling the remaining. This could lead to the generation of the putative 'carrier vesicles' of the stable compartment fame. These vesicles in turn undergo a second round of transformation (maturation) to become a compartment capable of fusion with a stable end state – the lysosomal system.

Late endosome to lysosome traffic: need for more trafficking models?

Although these studies have alluded above addressed the process of late endosome formation from early endosomes, they did not shed light on mechanisms of membrane trafficking from late endosomes to lysosomes. Late endosomes might mature to eventually become lysosomes or vesicular intermediates could be derived from late endosomes to deliver contents to lysosomes. There are other suggested mechanisms by which membrane trafficking between late endosomes and lysosomes could happen. They are the "kiss and run" model (Storrie and Desjardins 1996) or by formation of hybrid organelle [Fig. 5, (Bright et al. 1997)]. According to "kiss and run" model, late endosomes and lysosomes exhibit a variable, intrinsic ability to transiently fuse with each other. These transient connections result in establishment of size-selective exchange of materials between late endosomes and lysosomes. Experiments done on RAW 264.7 cell line (Duclos et al. 2003) or murine bone marrow derived macrophages (Berthiaume et al. 1995) showed that dextrans of various sizes, co-internalized in macrophages were rapidly segregated into distinct populations of endosomes. It was hypothesized

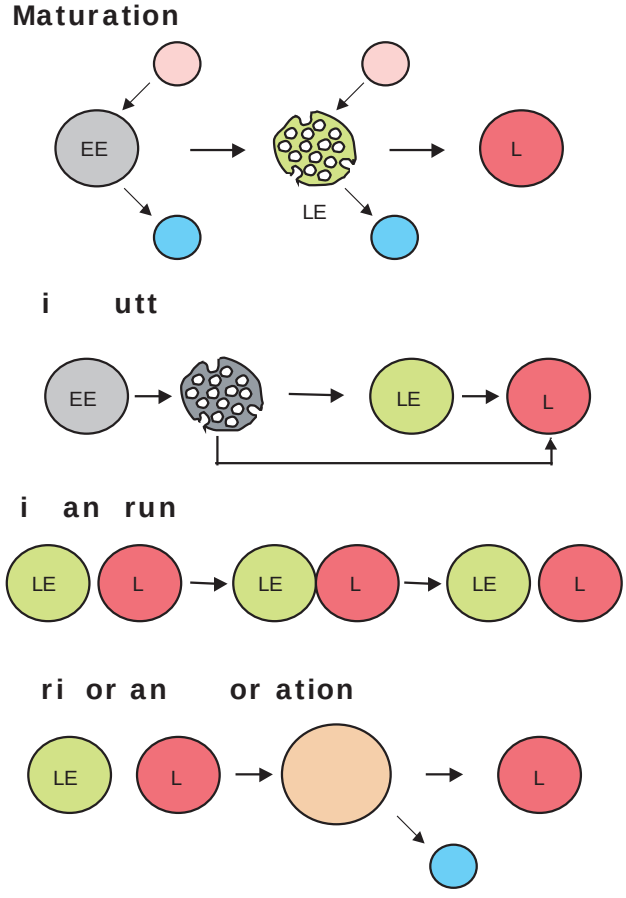

Figure 5: *Models of membrane trafficking involved in lysosome biogenesis*
A) Maturation model suggests that early endosomes (EE) are formed de novo *by fusion of vesicles formed via multiple pathways of endocytosis from the plasma membrane. Early endosomes mature to late endosomes (LE) and then to lysosomes (L) through the addition of TGN-derived cargo vesicles (CV) and removal of macromolecules in recycling vesicles (RV) destined for other compartments or plasma membrane. B) Vesicle shuttle model suggests that EE, LE and lysosomes are distinct pre-existing stable compartments. Macromolecules delivered to EE targeted for late endosomal delivery are transported in endosomal carrier vesicles that have multi-vesicular morphology to LE or directly to lysosomes. C) In the kiss and run model, maintenance and biogenesis of lysosomes occurs through LE-lysosome contact (kiss) with an accompanying transfer of contents and dissociation (run). D) In a variation of kiss and run model, LE and lysosomes undergo heterotypic fusions resulting in a hybrid organelle. Lysosomes are recovered from hybrid organelle, a step that involves condensation of contents into lysosomes and removal of endosomal macromolecules via degradation or recycling in hybrid organelle*

that this size-selective transfer reflected the occurrence of fusion events between endosomes containing the small and large tracers with endosomes devoid of tracer.

An extension of the "kiss and run" hypothesis is the formation of hybrid organelle where some kisses between late endosomes and lysosomes result in fusion. Hybrid organelles serve as compartments where degradation of endocytosed material would take place and from which lysosomes could be reformed. Experiments on cultured HEp-2 cells by Futter et al. (1996) showed that late endocytic degradative compartments (MVBs) accessible to epidermal growth factor receptor directly fused with M6PR-negative lysosomes (Futter et al. 1996). The authors pre-labeled these lysosomes using 15 min pulse of HRP followed by a 4 h chase. Activation of the lysosomal HRP by diaminobenzidine and H_2O_2 prior to epidermal growth factor (EGF) uptake prevented fusion of lysosomes with MVBs, but not maturation of the latter or docking of the MVBs with lysosomes. These experiments suggested direct fusion between MVBs and lysosomes. Experiments done in rat liver cell-free system by Mullock BM et al. (1998) using late endosomes loaded with avidin-asialofetuin and lysosomes loaded with ^{125}I-labeled biotinylated polymeric IgA showed that the immuno-precipitable product of content mixing between late endosomes and lysosomes was present in a membrane-bound organelle whose density was intermediate between that of late endosome and lysosome (Mullock et al. 1998). This organelle was termed a late endosome-lysosome hybrid organelle and contained markers of both lysosomes (cathepsin L) and late endosomes (endocytosed avidin-labeled asialofetuin, cation independent M6PR). The hybrid organelle had a mean diameter (0.96 mm) larger than those of either rat liver lysosomes (0.38 mm) or late endosomes (0.34 mm) and was less electron-dense than lysosomes (Mullock et al. 1998).

Once again it appears that *in vivo* imaging in the context of different molecular players, and their specific perturbation is necessary to provide insights into the complex process of endolysosomal biogenesis.

Function of endo-lysosomes

The endolysosomal system plays a crucial role in cellular homeostasis, being major sites for degradation of lipids and membrane proteins (Katzmann et al. 2002). Mutations resulting in defective function of lysosmal hydrolases results in many diseases.

Role of the acidified environment of endolysosomal system

As mentioned above, multiple, highly dynamic acidic organelles such as late endosomes and lysosomes with distinct morphologies constitute the late endosomal system. For the proper functioning of of the endo-lysosomal system, acidification of the lumen of this organelle is critical (Mellman et al. 1986). Vacuolar proton pumps (V-type H^+-ATPase) located in limiting endosomal membranes regulate the extent of acidification of late endosomes and lysosomes (Harikumar and Reeves 1983, Ohkuma et al. 1982, Schneider 1983). They use energy derived from ATP hydrolysis to power the translocation of protons (Forgac 1999). Endosomal acidification is partially regulated by reversible dissociation and reassembly of V-type H^+-ATPase complex and reversible disulphide bond formation between its different subunits (Forgac 1999). The extent of endosomal acidification in cells has been measured using lipophilic weak bases or selective labeling of compartments with endocytic tracers (Mellman et al. 1986). Lipophilic weak bases such as acridine orange or 9-amino acridine are membrane-permeant when uncharged at neutral pH and relatively membrane-impermeable once protonated. Thus, when allowed to equilibrate with either intact cells or isolated organelles, they accumulate within membrane vesicles that have acidic internal pH. The transmembrane pH gradient and total compartment volume decide the degree of accumulation of the probe (Glickman et al. 1983, Russell, 1984, Stone et al. 1983, Van Dyke et al. 1985). As compartment volume also decides the extent of accumulation of these probes in organelles, this method does not provide an accurate pH measurement.

pH probes introduced into endosomes or lysosomes by endocytosis have been used to measure the extent of compartment acidification with greater accuracy. For this purpose, fluorescein isothiocyanate-labeled dextran, a macromolecular marker of fluid-phase endocytosis has been used. Fluorescence intensity and excitation spectrum of fluorescein are titratable functions of pH (Ohkuma and Poole, 1978). Hence, standard curves can be constructed relating fluorescence intensity and compartment pH (Dunn et al. 1994). Using such measurements, pH in late endosomal lumen has been measured to be 5.2-5.8 (Yamashiro and Maxfield 1987) and lysosomal lumen has been measured as 4.6-5.0 (Geisow et al. 1981, Ohkuma and Poole 1978, Tycko et al. 1983).

Low pH in late endosomal compartments (Yamashiro and Maxfield 1987) facilitates the activity of lysosomal hydrolases, most of which have pH optima in the range of 4.5-5.5 (Mellman et al. 1986). It favours hydrolytic partly because acid-denaturation of many substrates makes them more susceptible to enzymatic degradation in lysosomes. The proton gradient drives coupled transport of lysosomal degradation products out of the lysosome as seen in transport of cysteine (Pisoni et al. 1985).

Acidification of the endosomal compartment may also facilitate traffic through the endolysosomal system. Disruption of late endosomal pH using lysosomotropic drugs results in endosomal accumulation of many integral membrane proteins such as asialoglycoprotein receptor (Strous et al. 1985, Tycko et al. 1983), low density lipoprotein receptor [LDLR, (Basu et al. 1981)] and M6PR (Gonzalez-Noriega et al. 1980, Reaves and Banting 1994, Tietze et al. 1982) as well as a defect in delivery of endocytosed HRP to lysosomes (van Weert et al. 1995). The reason for these defects is not clear. It could result from a massive redistribution of membrane in the cell and change in endo-lysosomal structure because of the lysomotrophic drugs used or by the alteration in pH-induced conformational change in structures of receptors/integral membrane proteins in endosomes somehow preventing their entry in to lysosome directed pathways (Mellman et al. 1986).

Control of duration and extent of signalling

Late endosomal system is involved in modulating duration of cell signaling; lysosomal delivery of signaling cell surface receptors has been proposed to be a negative feed back mechanism for down-regulating receptor signaling (Seaman et al. 1996). On ligand binding, activated EGFR (receptor tyrosine kinase; RTK) is endocytosed and sorted into lumenal vesicles of MVB destined for degradation while inactive EGFR remains on the limiting endosomal membrane (Felder et al. 1990, Futter et al. 1996, Hopkins et al. 1990). In endosomes, EGFR continues to recruit via its cytoplasmic domain, adaptor proteins that transduce the signal thus remaining signaling competent (Oksvold et al. 2001). *cbl*, a negative regulator of receptor tyrosine kinase signaling in *Drosophila* and *C. elegans* (Pai et al. 2000, Yoon et al. 1995) relocates from the cytosol to endosomes following EGFR internalization (Levkowitz et al. 1998, Meisner et al. 1997) and ubiquitinates EGFR. Ubiquitinated EGFR is sorted into MVBs and effectively sequestered away from cytosolic downstream signal transducing proteins as shown in figure 2. Tsg101 (human homologue of yeast class E *Vps23*) mutant fibroblasts are defective for sorting of endocytosed activated EGFR into lumenal vesicles of MVB. Hence the activated receptor is recycled and remains signaling competent causing a tumourogenic phenotype (Babst et al. 2000). *hrs* (*Drosophila* homologue of yeast *Vps27*) mutant larvae show defects in endosome membrane invagination and MVB formation. *hrs* mutant animals fail to degrade active EGF and Torso RTKs, leading to enhanced signaling and altered embryonic patterning (Lloyd et al. 2002).

Transforming growth factor β (TGF-β) receptors constitute a novel superfamily of single-pass transmembrane serine/threonine kinase receptors (Massague, 1992) that are degraded in lysosomes (Kavsak et al. 2000). E3 ubiquitin ligase Smurf is involved in ubiquitination of both TGF-β receptor and its effector Smad. Hrs binds ubiquitinated Smad and targets it into MVB (Miura et al. 2000). Consistent with this hypothesis, *Drosophila* Smurf mutant animals have an expanded Dpp (TGF-β homologue) gradient and fail to down-regulate Dpp signaling resulting in developmental defects (Podos et al. 2001).

Notch is another single-pass transmembrane protein that mediates many developmental signaling events including lateral inhibition and boundary specification (Ye and Fortini, 2000). Its ligands Delta and Serrate are transmembrane proteins resulting in juxtracrine signaling. Ligand binding results in cleavage at a distinct site of the extracellular domain of the receptor. This cleavage is followed by cleavage in the intracellular domain of Notch that translocates to the nucleus. Notch level in the cell is regulated by ubiquitin ligase Suppressor of deltex [Su (dx), (Cornell et al. 1999)] in *Drosophila*. Cell surface levels of Delta are regulated by its ubiquitin ligase Neuralized that promotes its internalization and degradation (Lai et al. 2001). Multivesicularization of endosomes thus regulates duration of receptor signaling in the cell, often by receptor specific mechanisms.

Establishment of morphogen gradients

The endolysomal system plays a crucial role in setting up morphogen gradients during development in *Drosophila*. Morphogens are secreted molecules that act in a concentration dependent manner to specify cell fates in a homogenous population of cells. Endosomal membrane trafficking has been demonstrated to be involved in setting up the concentration gradient and range of the morphogen not only by regulating the morphogens but also their down stream signaling factors. It has been demonstrated that a balance between degradation and recycling of rapidly endocytosed ligand bound to Thickvein, the receptor for the TGF-β family ligand, Decapentaplegic (Dpp) creates a Dpp gradient in the wing disc (Entchev et al. 2000). Over-expression of dominant active Rab7, that promotes early endosome to late endosome trafficking, results in reduced cellular Dpp and reduced domain of target Spalt expression consistent with decreased Dpp range (Entchev et al. 2000). Thus late endosomes sculpt the range of Dpp gradient.

Wingless glycoprotein is a morphogen that functions in a variety of developmental processes (Entchev and Gonzalez-Gaitan, 2002, Lawrence et al. 2002). Secreted wingless binds Frizzled receptor as well as extracellular matrix glycosaminoglycans. Wingless-HRP labels MVBs and MLEs in developing embryos indicating the

intracellular site of Wingless degradation (Dubois et al. 2001). It has been observed that zygotic mutants of *deep-orange* (*Drosophila* homologue of class C gene *Vps18*), defective in late endosomal trafficking show increased levels of Wingless in cells, enhancing the Wingless over-expression phenotype (Dubois et al. 2001). Thus, developing tissues seem to use late endosomal membrane trafficking to modulate morphogen gradients in time and space.

Role of efflux/recycling from the endolysosmal system

Multi-vesicular late endosomes/lysosomes fuse with the plasma membrane in many cell types resulting in secretion of small membrane vesicles referred as exosomes. Exosomes play a role in clearance of transferrin receptor, CD55, CD59 and integrin $\alpha_4\beta_1$ in reticulocytes (Denzer et al. 2000, Rabesandratana et al. 1998, Rieu et al. 2000). Cells of the hematopoietic system use MVBs to store and release their secretory products (Stinchcombe and Griffiths, 1999). Activated platelets secrete exosomes by fusion of adhesive glycoproteins containing alpha granules and MVBs with the plasma membrane (Heijnen et al. 1999). Antigen presenting cells have been shown to secrete exosomes that carry peptide loaded major histocompatability complex (MHC) molecules that stimulates T cell proliferation as discussed below (Fevrier and Raposo, 2004, Stoorvogel et al. 2002, Thery et al. 2002).

Lysosomes are involved in plasma membrane repair in fibroblasts. Damage to plasma membrane results in Ca^{2+} regulated fusion of lysosomes at the site of injury; a process regulated by lysosomal synaptotagmin VII isoform (Andrews, 2000, Reddy et al. 2001).

Antigen presentation

Loading of peptides onto MHC class II molecules for presentation of antigens to the immune system also take place in the endo-lysosomal system, and this is important in providing antigens from both endogenous petides and peptides derived from exogenous molecules to educate and sensitize the immune system (Janeway et al. 1996). This process takes place in professional antigen processing cells or APCs in the acidic lumen of lysosome-like organelle called MIIC (Neefjes 1999). MIIC is a pleiomorphic compartment present mainly in professional APCs such as macrophages and dendritic cells that expresses surface MHC class II-peptide complexes and enables activation of $CD4^+$ helper T-cells. In immature dendritic cells, 80% of MHC II molecules are localized in the luminal vesicles of multivesicular MIICs. The limiting membrane of MIICs is enriched for HLA-DM, chaperone-like protein that regulates the association of peptides with MHC class II molecules (Liljedahl et al. 1996, Sanderson et al. 1994, van Ham et al. 1997). In mature dendritic cells, the lumenal vesicles of the multivesicular MIICs fuse with the limiting membrane. This is accompanied by a dramatic morphological transformation of the MIIC resulting in its extensive tubulation followed by vesiculation (Kleijmeer et al. 2001). MHC II molecules are delivered to the cell surface from MIICs by these tubules and intermediate vesicles (Kleijmeer et al. 2001). These intermediate vesicles that deliver MHC II molecules to cell surface have been described as CIIVs (Turley et al. 2000). The endocytic pathway provides peptides derived from the degradation of antigens while the biosynthetic pathway provides newly synthesized MHC class II molecules . Peptides generated in the cytoplasm derived from endogenous and cytosolic protein sources, also gain access to MHC class II molecules in multivesicular late endosomes (Dani et al. 2004, Gueguen and Long 1996, Mukherjee et al. 2001). This may be important in the generation of self-tolerance in the context of CD4 positive cells. However, the mechanism of transport from the cytosol into the lumen of the late-endosome or lysosome is as yet unexplored.

Role of lysosome related organelles

In addition to the functions mentioned above, cell type specific lysosomes-like organelles perform specialized functions. Lysosome related organelles are usually cell type specific and more often than not, contain mature lumenal acid hydrolases and LAMP-1 on the limiting membrane. They also contain cell type specific components essential for their specialized function. They include melanosomes, lytic granules, platelet-dense granules, MHC II compartments, basophil granules, azurophil granules and pigment granules. The specialized functions of some of these lysosome related organelles is detailed below. Their function is severely affected by many human genetic lysosomal disorders further supporting a similarity between the cell type specific organelles and lysosomes.

Melanosomes generated within melanocytes are the site of synthesis and storage of a group of related pigments known as melanins. In mammals, these pigments determine the skin and hair color. In addition to lysosomal proteins they contain specific glycoproteins involved in melanin biosythesis namely tyrosinase, tyrosinase related protein 1 and 2 (Jackson 1988, Korner and Pawelek 1982, Tsukamoto et al. 1992) and are a major source of protection from UV radiation.

Drosophila pigment granules are membrane bound compartments that contain two pigments, brown ommochromes and red drosopterins in pigment cells of the compound eye. These pigment granules give *Drosophila* compound eye its intense red color. A

genetically amenable higher metazoan system that can be used to study membrane trafficking steps from which parallels in mammalian context can be drawn is *Drosophila*. Biogenesis of pigment granules in *Drosophila* compound eye is thought to be analogous to formation of lysosomes (Dell'Angelica et al. 2000). Genetic analysis suggests that biogenesis of pigment granules requires molecular components that play important role in protein delivery from TGN to lysosomes (Spritz, 1999). Thus, mutations affecting lysosome biogenesis could alter eye color in *Drosophila*. This connection between *Drosophila* eye color and lysosomal protein delivery was first established by identification of *Drosophila garnet* gene as the d-subunit of AP-3 complex (V. Lloyd, GenBank DMU31351). More than 85 mutations affecting *Drosophila* eye color have been isolated (Lindsley and Zimm 1992). Many of these genes code for enzymes necessary in the two distinct pigment synthesis pathways (such as *vermilion*, *cinnabar*) and ABC transporters likely to be involved in transfer of precursors of pigments across membranes (*white*, *scarlet*, *brown*). Rest of the genes constitute 'granule group' of eye color genes, mutants of which reduce both the chemically distinct pigments arguing against their role directly in pigment synthesis (Lloyd et al. 1998). The gene products are ubiquitously present and strong alleles cause sterility or lethality while weak alleles generate the characteristic eye color phenotypes. 'granule group' of eye color genes genetically interact with each other showing a variety of phenotypes not observed in the individual mutations including poor viability, sterility or synthetic lethality. *deep-orange* and *carnation*, *carnation* and *light*, *deep-orange* and *purploid* show synthetic lethal interactions (Lindsley and Zimm 1992, Lucchesi 1968). The recent molecular identification of 7 granule group genes (Table 4) has revealed a close relationship to genes involved in vacuolar trafficking in yeast and lysosomal trafficking in other organisms. Cellular assays studying lysosome biogenesis in eye color mutants of granule group genes could utilize this resource of putative molecular players involved in protein delivery to lysosomes for providing insights into molecular regulation of lysosomal membrane trafficking pathways.

Table 4:

Drosophila mutant	Mammalian orthologue	Yeast orthologue	Function of Yeast orthologue
Eye color	**Coat color**		
garnet	mocha	Alp5	δ-subunit of AP-3. ALP pathway
ruby	pearl	Alp6	β3 subunit of AP-3. ALP pathway
carmine	Ap-3μ	Apm3	μ3 subunit of AP-3. ALP pathway
orange	Ap-3σ	Aps3	σ3 subunit of AP-3. ALP pathway
light	mVps41	Vps41	Formation of AP-3 carrier vesicles
deep-orange	mVps18	Vps18	vacuolar protein trafficking
carnation	Buff	Vps33	vacuolar protein trafficking

Lytic granules aid in targeted secretion of macromolecules by cytotoxic T lymphocytes and natural killer cells used to destroy tumour or virus-infected cells. They are electron dense, endocytically accessible organelles that accumulate pore-forming perforin and granzymes (Clark and Griffiths, 2003). Their fusion with plasma membrane and release of contents is brought about by kinesin dependent movement along microtubules to the point of membrane contact with the target, a process triggered by increase in cytosolic calcium level (Griffiths and Argon, 1995, Lyubchenko et al. 2001).

Platelet dense granules in blood platelets play an important role in hemostasis and thrombosis. They contain a dense core of serotonin, calcium, ATP, ADP and pyrophosphate making them electron dense and include specific H^+ pump and serotonin transporter in the membrane (Dean et al. 1984). Elevation of cytosolic calcium levels results in secretion of dense granules, a process essential for the formation of the hemostatic plug (Yoshioka et al. 2001).

Lysosomes in disease

Lysosomes are a major site of intracellular catabolic processes. Many lysosomal storage disorders result form defects in specific catabolic enzymes that cause incomplete degradation of macromolecules. This leads to accumulation of metabolic intermediates in lysosomes (Gieselmann, 1995). In some of the sphingolipidosis class of lysosomal storage diseases such as Sandhoff disease, Fabry disease, Mucolipidosis or sphingolipid activator protein deficiencies, accumulation of the primary catabolic intermediate alters trafficking of sphingolipids, cholesterol and acid mucopolysaccharides (Pagano et al. 2000).

Diseases resulting from defects in lysosome biogenesis include Hermansky-Pudlak syndrome (HPS) that defines a group of autosomal recessive disorders characterized by defects in lysosome-related organelles. One of these, HPS type 2 results from a molecular lesion in β3A subunit of AP-3 adaptor protein complex. It is characterized by pigmentation defects caused due to defective melanosome biogenesis, immunodeficiency and bleeding diathesis (Huizing et al. 2000) resulting from defective platelet dense granules. The mutation results in mislocalization of lysosomal proteins to the plasma membrane (Aridor and Hannan 2000, Dell'Angelica et al. 1999, Huizing et al. 2000).

Chediak-Higashi syndrome is characterized by pigmentation defect and immunodeficiency. Lysosome and lysosome related organelle function is affected due to molecular lesion in LYST protein. The cellular function of LYST is not understood although domains of LYST show homology to proteins involved in intracellular trafficking (Aridor and Hannan 2000, Barbosa et al. 1996).

Griscelli syndrome (GS) is a rare autosomal recessive disorder affecting lysosome related organelles. It is characterized by pigment dilution of the hair (silvery hair), owing to the accumulation of pigment in melanocytes and reduced T-cell cytotoxicity caused by defects in cytolytic granule exocytosis (Menasche et al. 2000). Majority of GS patients studied have a defect in the *RAB27A* gene (Menasche et al. 2000). In the absence of Rab27a, melanosomes are unable to reach actin in the cell periphery, resulting in their net accumulation in the perinuclear region (Bahadoran et al. 2001). In cytotoxic T lymphocytes, cytolytic granules appear to retain the ability to polarize towards the target cell but are unable to fuse with the plasma membrane and kill target cells, suggesting that Rab27a regulates a late stage of granule release (Stinchcombe et al. 2001).

Danon disease is a neurological disease that also affects the heart and muscle caused by loss of LAMP-2. Loss of LAMP-2 results in likely loss of fusion between autophagic compartments and lysosomes and leads to development of a lysosomal glycogen storage disease as well (Nishino et al. 2000).

Niemann-Pick C (NPC1) is required for cholesterol transport from late endosomes and lysosomes to other cellular membranes. Mutations in NPC1 cause lysosomal lipid accumulation causing enlargement of liver and spleen and progressive neurological degeneration. NPC1 is a putative cholesterol transporter involved in vesicular trafficking of lipids from MVBs to Golgi (Liscum, 2000).

Budding of virus particles from cells infected with Human immunodeficiency virus-1 (HIV-1) uses the cellular MVB machinery. Topologically, the budding of an invagination into MVB and budding out of the plasma membrane to release the virus particle seem equivalent. The HIV-1 coat protein Gag is ubiquitylated and this ubiquitylation correlates with the efficient budding of viral particles (Ott et al. 2000, Schubert et al. 2000, Strack et al. 2000). It is likely that this ubiquitylation recruits cellular class E Vps proteins that are MVB associated. Virion assembly has been reported to occur in late endosomes (Pelchen-Matthews et al. 2003). Human homologues of class E Vps proteins such as Tsg101 have been found in HIV-1 particles. Dominant-negative mutants of human class E proteins have been shown to arrest HIV-1 budding from the plasma membrane and endosomal membranes. The proteins required for human MVB biogenesis seems to participate in the release of HIV and probably many other viruses (Garrus et al. 2001, Pornillos et al. 2003, von Schwedler et al. 2003).

Conclusion

Biochemical and genetic analyses of membrane trafficking to vacuole/lysosomes have resulted in identification of a slue of molecular players that are likely to be involved in setting up and regulating the endo-lysosomal system in yeast and higher metazoa. This catalogue of molecular players is likely to expand in the near future. At the level of the whole organism, role of endo-lysosomal system in development and disease is beginning to be appreciated. Membrane trafficking steps involved in lysosome biogenesis in higher metazoa that a large number of these molecular players regulate remain unclear. Visualizing lysosome biogenesis in live cells in context of these molecular players in a higher metazoan system would help in mapping the membrane trafficking steps involved in lysosome biogenesis that these molecules regulate. In this context, its is important to reflect that basic insights obtained from classic histochemical studies still provide a powerful framework to understand membrane trafficking in animal cells. It is also tribute to Alex Novikoff and co-workers (writing in early and late 70's), that many models of secretory lysosomal pathway were generated by studying the localization of histochemical markers of various organelles using high resolution offered by EM. In situ visualization will also allow understanding of the role of intracellular membrane trafficking in shaping tissue patterning during development. Mechanistic insights from such studies might further aid in understanding the process of animal development and disease.

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