Biochimica et Biophysica Acta 1793 (2009) 615–624

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Delivery of endocytosed membrane proteins to the lysosome

Paul R. Pryor [a,*], J. Paul Luzio [b,*]

[a] Department of Biology (Area 9), University of York, York, YO10 5YW, UK
[b] Cambridge Institute for Medical Research and Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0XY, UK

ARTICLE INFO

Article history:
Received 22 July 2008
Received in revised form 1 December 2008
Accepted 12 December 2008
Available online 8 January 2009

Keywords:
Endocytosis
Late endosomes
Lysosomes
Multivesicular body
Membrane fusion

ABSTRACT

The delivery of proteins from the plasma membrane to the lysosome for degradation is essential for normal cellular function. There is now a good understanding of the protein complexes involved in sorting proteins at the plasma membrane and into the intralumenal vesicles of the multi-vesicular body. A combination of cell free content mixing assays and live-cell imaging has dissected out the final step in delivery of macromolecules to the lysosome from the multi-vesicular body and provided insights into the molecular mechanisms by which late endosomes and lysosomes exchange lumenal contents. The endocytic pathway has provided a platform with which to understand the autophagic and phagocytic pathways, but the fine details of how traffic through these pathways is regulated remain to be determined.

1. Introduction

Lysosomes are highly dynamic membrane-bound organelles that act as the terminal degradative compartment of the endocytic, phagocytic and autophagocytic pathways (Fig. 1). Many of the proteins required for protein sorting and endocytic delivery to lysosomes in mammalian cells are orthologues of those used by the yeast *Saccharomyces cerevisiae* for delivery to the vacuole (the yeast equivalent of the lysosome). Studies on vacuolar delivery and fusion have been extremely informative in providing insights to the universal mechanisms governing sorting and delivery in eukaryotic cells. Additionally, some cell types contain lysosome-related organelles (LROs) that include melanosomes, lytic granules, MHC-II compartments, platelet dense granules and neutrophil azurophil granules [1]. Some LROs are simply the modified lysosome, for instance in cytotoxic T lymphocytes the lytic granules are the only lysosome-type organelle present. In other cell types such as melanocytes, both the melanosomes and the 'conventional' lysosomes co-exist. Studies of LROs, in particular from patients with Hermansky–Pudlak syndrome that have deficiencies in melanosomes and platelet-dense granules, have identified mutations in genes encoding proteins (some that are not found in yeast) that assemble into five distinct complexes (adaptor protein-3 (AP-3), homotypic fusion and vacuole protein sorting (HOPS) complex and biogenesis of lysosome-related protein complex (BLOC)-1, -2 and -3) that have provided further insights into delivery of endocytic cargo to the lysosome [2]. This review therefore summarises recent insights into endocytic sorting of plasma membrane proteins destined to be delivered to mammalian lysosomes. It includes descriptions of sorting at the plasma membrane and in endosomes and the plethora of proteins required to regulate and sort cargo into the intralumenal vesicles (ILVs) of multivesicular bodies (MVBs). Finally, as all membrane fusion events may be defined as having tethering and docking and fusion steps, the review will indicate the key regulators in the membrane fusion between MVBs and lysosomes.

2. Clathrin mediated endocytosis at the plasma membrane

A plasma membrane protein that is destined to be endocytosed needs to be recognised by the endocytic machinery. Although other uptake pathways have been described [3], the best understood endocytic mechanism is clathrin mediated endocytosis. At its simplest, this may be regarded as a co-ordinated series of events involving cargo recruitment into small regions of the plasma membrane, clathrin cage assembly, membrane bending, vesicle formation, vesicle fission and finally vesicle uncoating (for reviews see [4,5]). Cargo recruitment requires clathrin adaptors that interact with sequence motifs, structural features or added ubiquitin molecules in the cytosolic tail of the membrane protein. Some membrane proteins require the binding of extracellular ligand before rapid internalisation proceeds, but others do not need the addition of ligand. In the latter case, endocytosis occurs to maintain low levels of the protein at the plasma membrane and/or to redirect mis-sorted proteins to intracellular sites. Efficient incorporation of some endocytosed membrane proteins into clathrin- coated-pits and -vesicles (CCVs) is mediated by short linear amino acid sequences, such as the YXXΦ and [DE]xxxLL motifs, that are recognised by the clathrin adaptor AP2 (reviewed in [6–8]). Other membrane proteins, including several growth factor receptors, are

* Corresponding authors. P.R. Pryor is to be contacted at tel.: +44 1904 328563. J.P. Luzio, tel.: +44 1223 336780.
E-mail addresses: pp517@york.ac.uk (P.R. Pryor), jpl10@cam.ac.uk (J.P. Luzio).

0167-4889/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2008.12.022

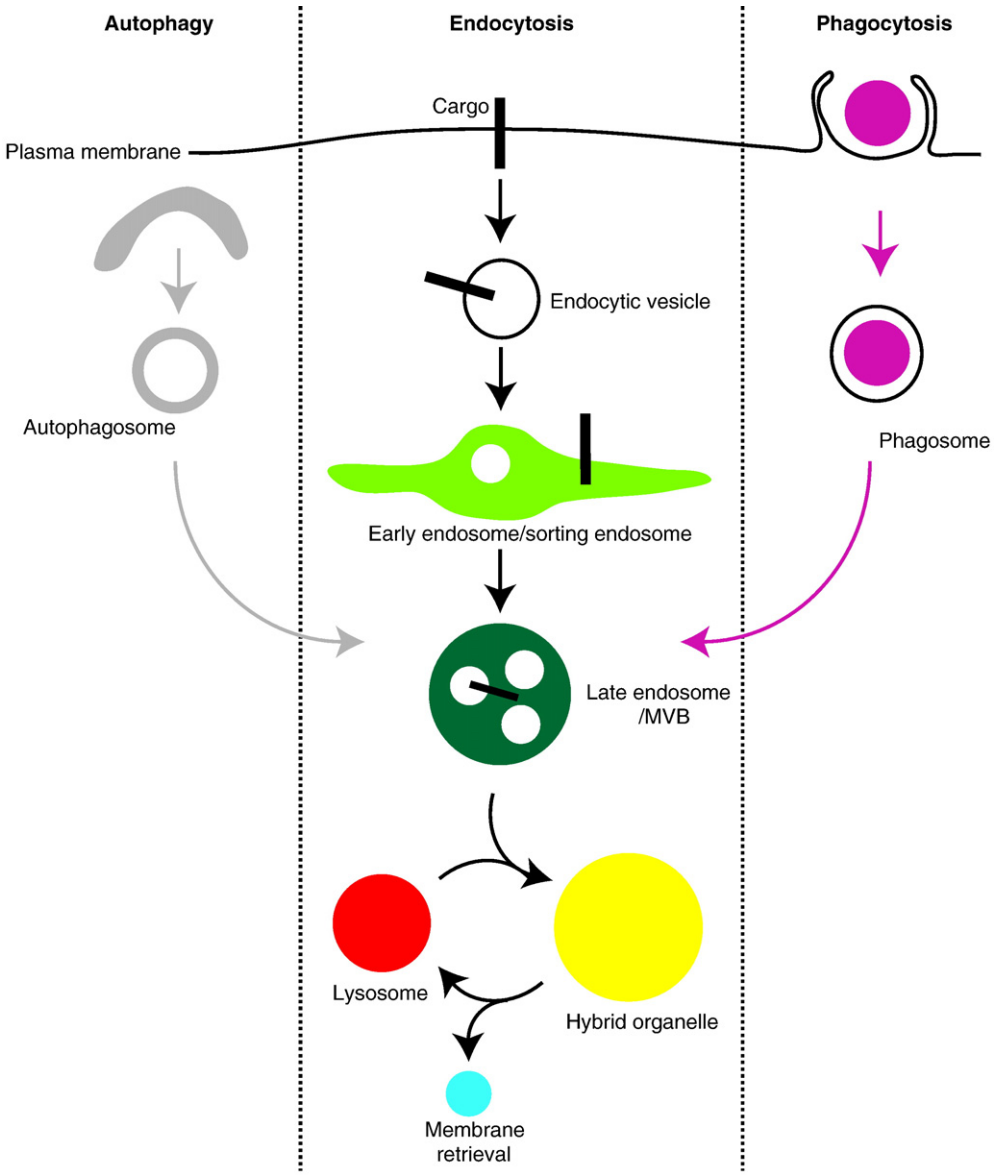

Fig. 1. Intracellular pathways to the lysosome. Endocytic cargo is internalised from the plasma membrane and delivered to early endosomes. Maturation of the early endosome gives rise to a late endosome/multi-vesicular body where the cargo destined for degradation has been sorted into intralumenal vesicles. The limiting membranes of late endosomes and lysosomes can fuse to form a hybrid organelle where degradation of endocytosed macromolecules commences. Lysosomes are reformed from the hybrid organelle by membrane retrieval and condensation reactions. Autophagic and phagocytic pathways both feed into the endocytic pathway with the lumenal contents of the autophagosome and phagosome eventually being delivered to the lysosome for degradation.

ubiquitylated, such that they carry multiple single ubiquitins (multiple monoubiquitylation), or short polyubiquitin chains, added to specific lysines in the cytosolic tail (reviewed in [9]). The presence of many ubiquitins allows efficient capture into CCVs by specific clathrin adaptors such as EPS15 and epsin, despite the relatively low affinity of ubiquitin binding domains in these adaptors for single ubiquitin molecules.

Recently, a third mechanism for efficient incorporation into CCVs has been described, and is used by at least one soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) at the plasma membrane. SNAREs are critical for driving membrane fusion events on the secretory and endocytic pathways (reviewed in [10]). However, the mechanisms by which SNAREs are actually sorted to different membranes are largely unknown. Most of the SNAREs do not possess short linear endocytic sequence motifs and are not known to be ubiquitylated. However, the folded N-terminal domain of the SNARE VAMP7 binds to a predicted unstructured region of the clathrin adaptor HIV Rev-binding protein (HRB) allowing endocytosis of VAMP7, but only when VAMP7 is incorporated into a cis-SNARE complex [11]. VAMP7 is found on lysosomes and is required for lysosome fusion with the plasma membrane. Its folded N-terminal domain thus acts as a retrieval signal from the cell surface and the interaction with an unstructured region of HRB has provided an insight into the potential role of the unstructured regions of clathrin adaptors in cargo recognition.

It should be noted that clathrin-mediated endocytosis may play an important role in lysosome biogenesis. In most cell types studied, many newly synthesised acid hydrolases are delivered to lysosomes *via* an intracellular route that requires addition of mannose 6-phosphate and binding to mannose 6-phosphate receptors, which traffic between the trans-Golgi network and endosomes. However, in proximal kidney tubule cells the lysosomal enzyme cathepsin B is delivered to lysosomes following secretion of non-mannose 6-phosphate-labelled pro-cathepsin B, that then binds to the cell surface