Endosome–lysosome fusion

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Abstract
The delivery of endocytosed cargo to lysosomes occurs through kissing and direct fusion of late endosomes/MVBs (multivesicular bodies) and lysosomes. Live-cell and electron microscopy experiments together with cell-free assays have allowed us to describe the characteristics of the delivery process and determine the core protein machinery required for fusion. The ESCRT (endosomal sorting complex required for transport) machinery is required for MVB biogenesis. The HOPS (homotypic fusion and vacuole protein sorting) complex is required for endosome–lysosome tethering and a trans-SNARE (soluble N-ethylmaleimide-sensitive factor-attachment protein receptor) complex including the R-SNARE VAMP7 (vesicle-associated membrane protein 7) mediates endosome–lysosome membrane fusion. Protein-binding partners of VAMP7 including the clathrin adaptors AP-3 (adaptor protein 3) and Hrb (HIV Rev-binding protein) are required for its correct intracellular localization and function. Overall, co-ordination of the activities of ESCRT, HOPS and SNARE complexes are required for efficient delivery of endocytosed macromolecules to lysosomes. Endosome–lysosome fusion results in a hybrid organelle from which lysosomes are re-formed. Defects in fusion and/or lysosome reformation occur in a number of lysosome storage diseases.

The mechanism and machinery of fusion
Lysosomes act as the terminal compartment of the endocytic and autophagic pathways (Figure 1). It is now recognized that the delivery of endocytosed and autophagocytosed macromolecules to lysosomes for degradation is mediated by kissing events and direct fusion of lysosomes with late endosomes/MVBs (multivesicular bodies) and autophagosomes respectively [reviewed in [1,2]]. In addition, lysosomes are able to fuse with the plasma membrane in response to insults to the cell surface that cause a rise in cytosolic Ca2+ concentration above 1 μM [3]. Evidence for the direct fusion of lysosomes with endosomes initially came from immuno-electron microscopy, compartment ablation studies and cell-free content mixing assays [reviewed in [2]]. This was extended by live-cell confocal microscopy experiments that showed that kissing events as well as direct fusion contributed to content mixing between these organelles [4,5]. Live-cell confocal microscopy experiments have also demonstrated that autophagosomes fuse with lysosomes [6].

Experiments using cell-free systems and transfected cultured cells have established the mechanistic steps involved in late endosome–lysosome fusion. Like other fusion events in secretory and endocytic membrane traffic pathways, endosome–lysosome fusion conforms with the tenets of the SNARE (soluble N-ethylmaleimide-sensitive factor-attachment protein receptor) hypothesis (reviewed in [7]). NSF (N-ethylmaleimide-sensitive factor) and soluble NSF-attachment proteins are required for fusion, with specificity provided by SNAREs and a small GTPase Rab protein (probably Rab7) with its associated effectors and tether proteins. For endosome–lysosome fusion, the composition of the tethers has not been formally established, but the mammalian HOPS (homotypic fusion and vacuole protein sorting) complex is a good candidate that interacts with Rab7 and syntaxin7 [8]. Consistent with this, overexpression of the mammalian HOPS complex components VPS (vacuolar protein sorting) 18 and VPS39 causes clustering of late endosomes and lysosomes [9,10], whereas depletion of VPS18 results in dispersion [10].

The trans-SNARE complex
Using antibody inhibition experiments in cell-free systems, it has been established that for both homotypic late-endosome fusions and heterotypic late-endosome–lysosome fusions, the same Qa-, Qb- and Qc-SNAREs

Key words: endocytosis, endosome, lysosome, multivesicular body (MVB).

Abbreviations used: BAPTA, 1,2-bis-(o-aminophenoxy)ethane-N,N',N''-tetra-acetic acid; CHMP, charged multivesicular body protein; EGTA-AM, EGTA acetoxymethyl ester; ESCRT, endosomal sorting complex required for transport; HOPS, homotypic fusion and vacuole protein sorting; ILV, intraluminal vesicle; MVB, multivesicular body; NSF, N-ethylmaleimide-sensitive factor; SNARE, soluble NSF-attachment protein receptor; VAMP7, vesicle-associated membrane protein 7; VPS, vacuolar protein sorting.

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Figure 1 | Lysosome fusion with other organelles
Outlines of the fusion of lysosomes with late endosomes, autophagosomes and the plasma membrane.

A requirement for Ca\(^{2+}\)?

A Ca\(^{2+}\) requirement for endosome–lysosome fusion has been reported from cell-free experiments [19,20]. Fusion was inhibited by BAPTA [1,2-bis-(o-aminophenoxy)ethane-N\(_2\)N\(_2\)N\(_{2}\)N\(_{2}\)-tetra-acetic acid] but not EGTA (which exchanges Ca\(^{2+}\) approx. 100-fold more slowly than BAPTA) and was also prevented by pre-incubation with a membrane-permeant ester, EGTA-AM (EGTA acetoxymethyl ester), which is cleaved within the organelle lumen, resulting in chelation of luminal Ca\(^{2+}\). The inhibitory effects of BAPTA and EGTA-AM were reversed by the addition of CaCl\(_2\). These data strongly support a role for the release of Ca\(^{2+}\) from the endosome lumen in fusion events in the endocytic pathway. Experiments in which increasing concentrations of CaCl\(_2\) were added to fusion assays in the presence of BAPTA suggested that 0.5 μM Ca\(^{2+}\) is optimal for fusion of late endosomes with lysosomes [19], very similar to 0.3 μM Ca\(^{2+}\) that is optimal for early-endosome fusion [21], but significantly less than the 1 μM, or higher, concentration required for lysosome fusion with the plasma membrane. Indeed, concentrations of Ca\(^{2+}\) of 0.4 μM and above have been reported to inhibit homotypic early-endosome fusion [21] and above 1 μM to inhibit heterotypic fusion of late endosomes with lysosomes [19]. Given these observations, it seems unlikely that synaptotagmin VII, which has been shown to be localized to lysosomes and to play a key role in Ca\(^{2+}\)-stimulated fusion of lysosomes with the plasma membrane, [22], is similarly required for fusion events in the endocytic pathway including endosome–lysosome fusion, although this has not been formally tested. What has been shown in a cell-free assay for endosome–lysosome fusion is that the BAPTA effect occurs later in the docking/fusion process than inhibition by Rab-GDI (GDP-dissociation inhibitor) [19]. Indeed, it may, as in yeast vacuole fusion, occur after trans-SNARE complex formation [23]. The use of calmodulin antagonists has suggested that calmodulin may mediate the Ca\(^{2+}\) requirement for endosome–lysosome fusion, but other mediators are possible. In this context, it is interesting to note the evidence that the penta-EF-hand protein ALG-2 (apoptosis-linked gene 2) binds to the N-terminal cytosolic tail of the lysosomal cation channel mucolipin-1 in a strictly Ca\(^{2+}\)-dependent manner [24]. This interaction modulates the function of mucolipin-1 and may play a role in endosome-lysosome fusion and/or the re-formation of lysosomes from the hybrid organelles formed when endosomes fuse with lysosomes. The process of re-formation of lysosomes has been shown to require luminal Ca\(^{2+}\), as well as a proton-pumping ATPase, in a cell-free system [19]. Although mucolipin-1 is a lysosomal cation channel that may be important in facilitating the Ca\(^{2+}\) requirement for endosome–lysosome fusion and/or lysosome re-formation, other channels may also be required, notably the lysosomal two-pore channels that are highly selective for Ca\(^{2+}\) [25,26]. Abnormalities in lysosomal Ca\(^{2+}\) may underlie trafficking defects observed in some lysosomal storage diseases [27]. Cells from patients with Niemann–Pick type C1 disease have reduced lysosomal Ca\(^{2+}\) stores that result in a block in endocytic traffic to lysosomes, and compensating for the reduced Ca\(^{2+}\) concentration reverses the phenotype [28]. Whereas the integral membrane Niemann–Pick type C protein NPC1 has been proposed to be required
for late-endosome–lysosome fusion, the soluble lysosomal Niemann–Pick type C protein NPC2 may play a role in re-formation of lysosomes from hybrid organelles [29].

The endosomal fusion partner for endosome–lysosome fusion

Endocytosed cargo that is being delivered to lysosomes first passes through early and late endosomes. Consistent with this pathway, cell-free experiments have demonstrated that lysosomes fuse much more efficiently with late endosomes than with early endosomes [30]. The formation of late endosomes from the dynamic early-endosomal network requires Rab conversion, with the endosomes losing Rab5 and acquiring Rab7. This process of Rab conversion requires the HOPS complex and the associated protein SAND-1/Mon1 [31,32]. This requirement for HOPS is additional to its role in tethering late-endocytic organelles before fusion. Late endosomes are synonymous with MVBs and contain many ILVs (intraluminal vesicles) formed through the recruitment and action of the ESCRT (endosomal sorting complex required for transport) proteins. However, in addition to the formation of ILVs and the sorting of ubiquitinated cargo into them, the ESCRT proteins CHMP (charged multivesicular body protein) 2B, CHMP3 and CHMP5 have also been implicated as having a role in endosome–lysosome fusion [33–35]. It has been suggested that mutations in CHMP2B that result in frontotemporal dementia act to inhibit this fusion [35]. How a late-acting function of ESCRT proteins in endosome–lysosome fusion is mediated is presently unclear, but it is interesting to note that there have been reports of interactions between ESCRT and HOPS proteins (discussed in [36]) that might imply a mechanistic involvement in tethering.

Conclusions

We now have a reasonable overview of the kissing, fusion and lysosome re-formation events required for delivery of endocytosed macromolecules to lysosomes. The core protein machineries to achieve this delivery have been described. However, we have little understanding of how these core machineries are regulated and co-ordinated. Moreover, we are probably only at the start of understanding how these machineries become disrupted to cause ‘traffic jams’ and pathological consequences in lysosomal storage and other diseases.

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References


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