Lysosomes: fusion and function

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Abstract | Lysosomes are dynamic organelles that receive and degrade macromolecules from the secretory, endocytic, autophagic and phagocytic membrane-trafficking pathways. Live-cell imaging has shown that fusion with lysosomes occurs by both transient and full fusion events, and yeast genetics and mammalian cell-free systems have identified much of the protein machinery that coordinates these fusion events. Many pathogens that hijack the endocytic pathways to enter cells have evolved mechanisms to avoid being degraded by the lysosome. However, the function of lysosomes is not restricted to protein degradation: they also fuse with the plasma membrane during cell injury, as well as having more specialized secretory functions in some cell types.

Endosome
A membrane-bound compartment (organelle) to which ligands, membrane components and fluid are delivered following internalization (endocytosis) from the cell surface.

Membrane whorl
A membranous structure that has the appearance of being multi-lamellar or arranged in spirals when observed in cross-section.

Autophagy
A process by which cytoplasmic components, including organelles, can be sequestered into autophagosomes and subsequently degraded.

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Lysosomes are membrane-bound organelles that are present in animal cells and contain acid hydrolases. They can be distinguished from endosomes by the lack of mannose-6-phosphate receptors (MPRs). Lysosomes were discovered by Christian de Duve more than 50 years ago as a result of studying the intracellular distribution of enzymes using centrifugal fractionation[1] (BOX 1). Electron microscopy, which was not used in their initial discovery, subsequently showed that lysosomes constitute up to 5% of the intracellular volume and are of heterogeneous size and morphology; they often contain electron-dense deposits and membrane whorls[2]. Within a few years of their discovery, lysosomes were recognized as the terminal degradative compartment of the endocytic pathway; they are also required for the digestion of the intracellular material that is segregated during the process of autophagy[3]. Lysosomes were shown to have proton-pumping vacuolar ATPases, which maintain the lumenal environment at a pH of 4.6–5.0 (REF. 4).

Once endosomes were also identified[5], it became well established that many endocytosed macromolecules, such as low-density lipoprotein, are delivered to lysosomes after their sequential passage through early and late endosomes[6,7]. However, the mechanism of transfer of endocytosed material from endosomes to lysosomes remained controversial, with several theories being proposed (FIG. 1a). These included maturation (of the endosome into a lysosome), vesicular transport (with vesicles carrying cargo from endosomes to lysosomes), kiss-and-run (a continuous cycle of transient contacts or 'kisses' between endosomes and lysosomes, during which material is transferred between the organelles and each contact is followed by a dissociation or 'run'), direct fusion (of the endosome to the lysosome to form a hybrid organelle) and fusion–fission (a variation of direct fusion and kiss-and-run, in which lysosomes re-form from hybrid organelles as a result of fission events)[8–11]. Recently, time-lapse confocal microscopy experiments have shown that kissing and direct fusion events both contribute to the mixing of the contents of endosomes and lysosomes in living cells[12]. These experiments, together with earlier electron-microscopy studies and the investigation of cell-free interactions of endosomes and lysosomes, have resulted in a much greater understanding of the dynamics of late endocytic organelles. The fusion of endosomes with lysosomes creates hybrid organelles in which the bulk of the endocytosed cargo is degraded. Reformation of lysosomes from these hybrid organelles requires retrieval and/or recycling of some membrane proteins by vesicular traffic[12,13]. This view of lysosomes as fusogenic organelles is consistent with other data showing that lysosomes can also fuse with phagosomes, autophagosomes and the plasma membrane under appropriate circumstances (FIG. 1b). Here, we summarize the routes of membrane traffic to lysosomes and discuss our current understanding of the mechanism by which lysosomes fuse with other organelles. The function of these fusion events is discussed, along with the mechanisms by which phagocytosed microorganisms may evade delivery to lysosomes.

Membrane traffic routes to lysosomes

Considerable information is now available concerning how newly synthesized hydrolases and membrane proteins are delivered from the *trans*-Golgi network (TGN) to lysosomes in mammalian cells. Many of the proteins in the mammalian trafficking machinery are orthologues of those used by *Saccharomyces cerevisiae* for delivery from the Golgi to the vacuole (the yeast equivalent of the mammalian lysosome)[10,11,14].

Box 1 | **The discovery of lysosomes**

The discovery of lysosomes more than 50 years ago was a triumph for subcellular fractionation using strictly biochemical criteria[1]. During *in vitro* assays of enzymatic activity, it was observed that acid phosphatase and some other enzymes were more active after subcellular fractionation or in homogenates of rat liver that were left in the refrigerator for several days, which suggested latent enzymatic activity. Many conditions were used to release the latency, including mechanical disruption, freeze–thawing, osmotic activation and, later, detergents. The latent enzymatic activity was explained when electron microscopy studies subsequently showed that lysosomes were subcellular organelles surrounded by a membrane that prevented the substrate and the enzyme from mixing in the assays that were carried out. The early experiments of de Duve and his collaborators were also important for other reasons. The researchers reported subcellular fractionation by plotting the relative specific activity of an enzyme against protein concentration in the subcellular fraction. This rapidly allowed them to allocate enzymes to different subcellular fractions and to establish the postulates of biochemical homogeneity and unique localization. These state that every type of cytoplasmic organelle or particle has a common biochemical composition, and that specific enzymes are found in a single class of particle or organelle. Although these postulates are now recognized as approximations, they continue to be of importance in assessing the purity of subcellular fractions. The first international symposium on lysosomes was held in London, UK, in 1963. The record of that meeting[3] is important, not only because it brought together what was known about lysosomes at that time but also because, in a footnote on page 126 of the meeting report, de Duve first proposed the use and definition of the terms 'endocytosis' and 'exocytosis'.

Delivery of lysosomal proteins to lysosomes. In mammalian cells, many newly synthesized acid hydrolases are delivered to lysosomes after they are tagged with mannose-6-phosphate in the *cis*-Golgi and subsequently bind to MPRs in the TGN. The bound hydrolases are first delivered to endosomes, where they dissociate from the receptors as a result of the acidic lumenal pH; this allows the receptors to recycle back to the TGN[15] and the hydrolases continue onwards to lysosomes. Clathrin, its heterotetrameric adaptor AP1 (adaptor protein-1) and the monomeric adaptors known as GGAs (Golgi-localized, γ-ear-containing, ADP ribosylation factor-binding proteins) are required for MPR trafficking from the TGN to endosomes[16,17].

Unlike soluble hydrolases, the delivery of newly synthesized lysosomal membrane proteins from the TGN does not require their binding to MPRs, and occurs either by an indirect route via the plasma membrane or by a direct intracellular route. The best-studied direct route is that requiring the heterotetrameric adaptor AP3 (adaptor protein-3) (REFS 18,19), although this can also function in the indirect route because its major site of action is at tubular endosomes. Mammalian protein complexes that do not have yeast orthologues, such as the biogenesis of lysosome-related organelles complex-1 (BLOC1), might also be involved in trafficking by interacting with AP3 (REFS 20,21). One lysosomal membrane protein, lysosome-associated-protein transmembrane-5 (LAPTM5), has been shown to traffic directly from the TGN to lysosomes as a result of an association with a ubiquitin ligase and GGA3, although LAPTM5 is not itself ubiquitinated[22]. All newly synthesized lysosomal proteins, whether trafficking directly or indirectly to endosomes, use a common delivery pathway from late endosomes to lysosomes.

Delivery of endocytosed membrane proteins. Some endocytosed ligands, such as low-density lipoprotein, dissociate from their receptors in the acidic lumen of the early endosome and are delivered to the lysosome for degradation. This process also allows subsequent recycling of the empty receptors to the cell surface. Other ligands, such as epidermal growth factor (EGF), remain bound to their receptors (which are ubiquitylated[23,24]) and, after endocytosis, the receptor–ligand complexes are internalized from the surface of the endosome into lumenal vesicles. Late endosomes containing these lumenal vesicles fuse with lysosomes.

The mechanism by which late endosomes are formed from early endosomes has been the subject of dispute with two models emerging: a maturation model[25,26] and a model in which endocytic carrier vesicles[27], formed as a result of the recruitment of cytosolic coat proteins to early endosomes, are required for the transfer of cargo to late endosomes. Recent live-cell imaging studies have reconciled mechanistic aspects of both models, with the observation that large (400–800 nm) vesicles arise from a dynamic early endosome network and undergo a conversion in which they lose the small GTPase RAB5 and recruit RAB7 (REF. 28). Late endosomes contain more lumenal vesicles than early endosomes and are often described as multivesicular bodies (MVBs). The lumenal membrane vesicles are enriched in either lysobisphosphatidic acid or phosphatidylinositol 3-phosphate[29]. There is evidence that not all MVBs are functionally equivalent. For example, EGF and the EGF receptor (EGFR) traffic through a subpopulation of MVBs that are morphologically identical to other MVBs but are distinguished by containing annexin-1 and lacking lysobisphosphatidic acid[30].

The mechanism by which MVBs are formed and the sorting of ubiquitylated membrane proteins into their lumenal vesicles has been the subject of much recent investigation[31–35]. In yeast, 12 soluble vacuolar protein sorting (Vps) proteins organize into four ESCRT complexes (endosomal sorting complexes required for transport) — ESCRT-0, -I, -II and -III — that are required for protein trafficking to the vacuole. Additional soluble Vps proteins that are associated with ESCRT-III are also important for this process. Current models suggest that ESCRT-0 binds to ubiquitylated cargo proteins and recruits ESCRT-I, which, in turn, recruits ESCRT-II

Phagosome
A membrane-bound compartment containing particles such as bacteria, yeast or parasites that have been internalized from the cell surface by the process of phagocytosis.

Autophagosome
An intracellular compartment that is formed when a double membrane sequesters a portion of cytoplasm that often includes organelles. Autophagosomes subsequently fuse with lysosomes.

trans-Golgi network
A tubulovesicular structure on the *trans* side of the stack of Golgi cisternae where cargo molecules are sorted to different secretory destinations.

Multivesicular body
An endosome that contains many vesicles in the lumen of each organelle. It can also be called a late endosome.

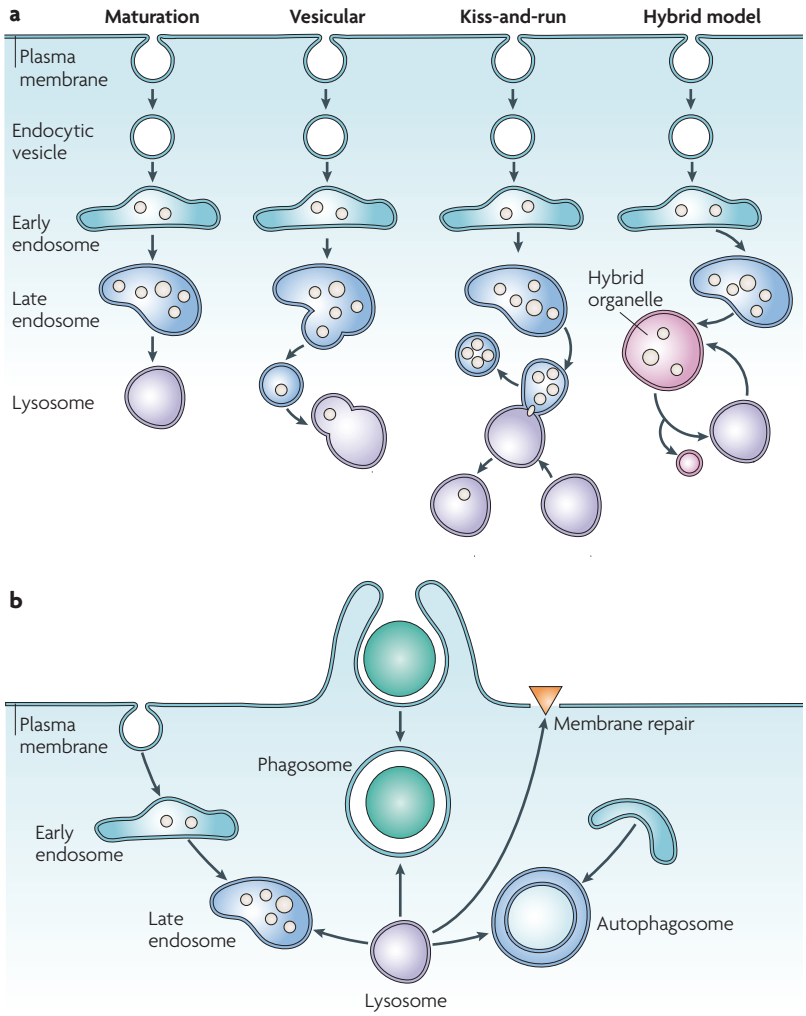

Figure 1 | **Delivery to lysosomes and lysosomal fusion. a** | Endocytic cargo is internalized from the plasma membrane first to early endosomes and then to late endosomes. Late endosomes deliver their cargo to lysosomes where the cargo molecules are degraded. Different models have been proposed to explain how cargo is trafficked from late endosomes to lysosomes. In the first model (maturation), late endosomes mature into lysosomes by the gradual addition of lysosomal components and removal of late endosome components. In a second vesicular model, vesicles may bud from the late endosome that delivers its contents to the lysosome. In the third model, late endosomes and lysosomes may transiently fuse (kiss), allowing for the exchange of contents between them, before departing again (run). In the final model (hybrid), endosomes and lysosomes may permanently fuse to form a hybrid organelle that contains both lysosome and late endosome components. Lysosomes are then re-formed by the selective retrieval of late endosome components. **b** | Lysosomes can fuse with different cellular membranes: with endosomes, autophagosomes, phagosomes and the plasma membrane (for the purpose of membrane repair).

and ESCRT-III. Cargo is deubiquitylated and the ESCRT complexes are removed from the membrane, which allows cargo to be sorted into the inward budding vesicle. Many details of the ESCRT pathway remain to be established in yeast; new components continue to be found[36–39] and the mechanism of inward budding remains to be clarified.

In mammalian cells, the recruitment, assembly and disassembly of ESCRT complexes is thought to be similar to yeast, but is probably more complex, as suggested by the existence of multiple homologues of some of the unique yeast Vps proteins. Recruitment of the ESCRT machinery and the initial formation of lumenal vesicles occurs at early endosomes but continues in late endosomes. Some endocytosed, ubiquitylated cargoes apparently do not require all of the ESCRT complexes for lysosomal degradation in mammalian cells, which implies the existence of cargo-specific ESCRT sorting pathways[40,41].

One interesting question is whether the correct functioning of the ESCRT pathway is necessary for subsequent fusion events with lysosomes. In cultured cells, depletion of the human ESCRT-III protein VPS24 by RNA interference results in the accumulation of EGFR in MVBs[42]. This was interpreted as being the result of inhibited fusion between MVBs and lysosomes, which implies a function for mammalian VPS24 that is distinct from its role in MVB biogenesis and sorting. Other published data also hint that an endosome must be appropriately prepared to fuse with a lysosome[28,43]. In cell-free systems, little or no fusion has been observed between early endosomes and either late endosomes or lysosomes[44,45].

Endosome fusion with lysosomes

Although lysosome fusion events (particularly those with phagosomes) were described in early work[2,3], it required the advent of new techniques, including immunoelectron microscopy and data from cell-free content-mixing assays, to provide good evidence that late endosomes or MVBs fuse directly with lysosomes[45–48]. Much of this fusion occurs in the juxtanuclear region of the cell because late endosomes and lysosomes are concentrated near the microtubule-organizing centre. The organelles are often observed in close proximity by electron microscopy (FIG. 2a). It has only recently been possible to address how endocytosed macromolecules are delivered to lysosomes in living cells.

Bright *et al.* used live-cell confocal microscopy to show that content mixing occurred between endosomes and lysosomes in normal rat kidney (NRK) fibroblast cells, and that endosome–lysosome fusion had several characteristics[12]. First, content mixing was only observed when the organelles were in physical contact: vesicle-mediated trafficking between organelles was not observed. Second, organelles either transiently fuse (kissing events) or undergo permanent fusion. Kissing events often, but not always, precede full fusion. Third, contents were sometimes exchanged between organelles by tubules that occurred from either type of organelle. Tubules can facilitate the exchange of contents between organelles by both kissing and full fusion events[12]. Using correlative live-cell and electron microscopy, images have been captured at the point of fusion, which show electron-dense lysosomal content diffusing into the endosome lumen[12] (FIG. 2b). (For schematic animations of direct fusion and 'kiss-and-run' events, see the lysosome–endosome interactions web page.)

Live-cell studies, together with studies in cell-free systems and transfected cultured cells, have established the protein machinery and mechanistic steps that are

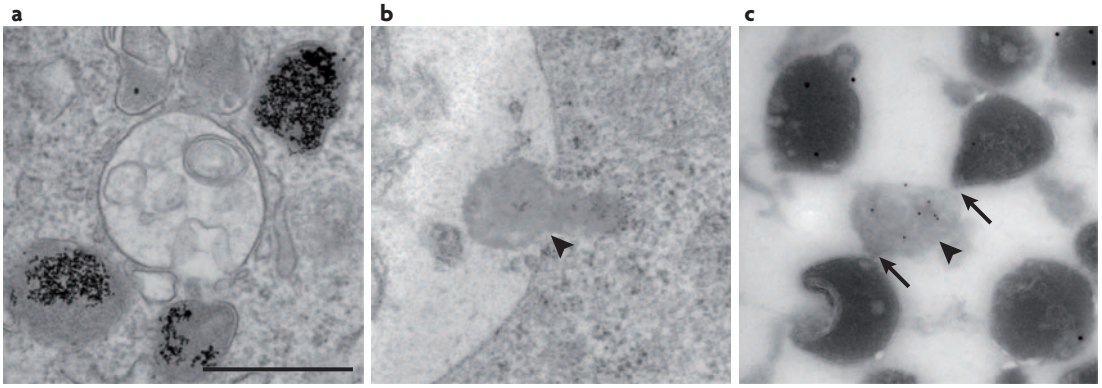

Figure 2 | **Electron microscopy of endosome–lysosome fusion. a** | Dense-core lysosomes in normal rat kidney (NRK) cells were loaded with colloidal gold conjugated with bovine serum albumin for 4 h followed by a 24 h chase. The lysosomes (dark grey) can be compared with a less-dense late endosome in the centre of the image. **b** | An electron-dense lysosome (arrowhead) in an NRK cell is captured in the process of fusing directly with an electron-lucent endosome by correlative live-cell imaging and electron microscopy. The image shown is from a 50 nm serial section immediately adjacent to that shown in Bright *et al.* (REF. 12). **c** | Immunogold electron microscopy of dense lysosomes from a rat liver preparation (labelled with a cathepsin D lysosomal marker; 15 nm gold). Lysosomes were isolated following an *in vitro* content-mixing assay[48]. The image shows that multiple lysosomes can form robust attachments (outer arrows) with an endosome (central arrowhead). The endosome was loaded with asialofetuin–avidin for 6 min and was subsequently immuno-labelled with 10 nm colloidal gold. The scale bar in part **a** is 500 nm; parts **b** and **c** are the same scale as part **a**.

involved in the fusion of late endosomes with lysosomes (FIG. 3). In common with other fusion events in the secretory and endocytic pathways, the fusion of late endosomes and lysosomes requires the presence of *N*-ethylmaleimide sensitive factor (NSF), soluble NSF attachment proteins (SNAPs) and a small GTPase of the Rab family, probably RAB7 (REF. 48). Similar to other fusion events, the process can be considered as having three sequential steps: tethering, the formation of a *trans*-SNARE (SNAP receptor) complex that bridges across the two organelles and membrane fusion.

Tethering. A prerequisite to organelle fusion is organelle tethering, whereby two organelles form links between each other that extend over distances of >25 nm from a given membrane surface. The physical existence of tethers between late endocytic organelles was implied by morphological observations of fine striations between adjacent late endosomes and lysosomes in cultured cells[46,47,49], as well as by the ability to show endosome–lysosome interactions in cell-free systems[50] (FIG. 2c). The composition of the tethers has not been established but the mammalian homotypic fusion and vacuole protein sorting (HOPS) complex, which is recruited by RAB7, is a good candidate (FIG. 3a). Overexpression of the mammalian HOPS complex components VPS18 and VPS39 caused clustering of late endosomes and lysosomes[51,52] and depletion of VPS18 resulted in organelle dispersion[52]. However, it is clear that these components are not specific for heterotypic late endosome–lysosome fusion and that they also function in homotypic endosome fusion even in the early part of the endocytic pathway[53]. Overexpression of RAB7 and some RAB7 effectors can also cause clustering of late endocytic organelles[54,55], and dominant-negative mutants of RAB7 cause dispersion[54].

Dominant-negative mutant
A protein encoded by a mutated gene that prevents the function of the wild-type protein in cells in which both the mutant and wild-type proteins are expressed at the same time.

It should be noted that tethering is a separate process from that which causes the accumulation of late endosomes and lysosomes in the juxtanuclear region around the microtubule-organizing centre, although this might also increase the efficiency of delivery of endocytosed macromolecules to lysosomes. Juxtanuclear accumulation reflects the balance of long-range bidirectional movement on microtubules and short-range movement on actin filaments. Such movement is mediated by motor proteins and proteins that are required for the optimal attachment of these motors to late endocytic organelles, including the RAB7 effector RAB7-interacting lysosomal protein (RILP)[56] and BLOC3 (REF. 57).

Trans-SNARE complex formation. Following tethering, a *trans*-SNARE complex must form in which the ~16-turn helix of one SNARE wraps around similar helices on three other SNAREs to form a parallel four-helix bundle called a SNAREpin, which is essential for membrane fusion[58]. The centre of the four-helix bundle contains an ionic layer comprising an arginine (R) and three glutamine (Q) residues, each contributed by a different SNARE. These residues are termed R-SNARE and Qa-, Qb- and Qc- SNAREs, respectively[59,60]. A functional *trans*-SNARE complex must contain one helix of each type[61]. Antibody-mediated function-blocking experiments in cell-free systems have provided the most compelling evidence that the same Qa, Qb and Qc SNAREs — syntaxin-7, VTI1B (VPS10 tail interactor-1B) and syntaxin-8, respectively — are required both for homotypic late endosome fusions and heterotypic late endosome–lysosome fusions[62–64]. What distinguishes the two fusion events is the R-SNARE, which is vesicle-associated membrane protein-8 (VAMP8) for homotypic late endosome fusion, and VAMP7 (also known as

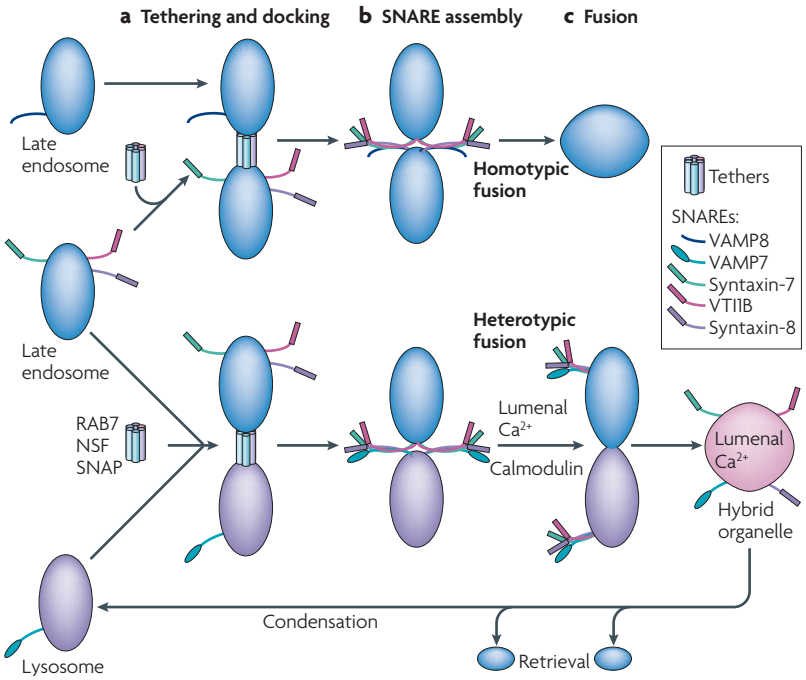

Figure 3 | **Schematic models of heterotypic late endosome–lysosome fusion and homotypic late endosome fusion. a** | The small GTPase RAB7, possibly in conjunction with the mammalian homotypic fusion and vacuole protein sorting (HOPS) complex, is thought to tether endosomes and lysosomes (or endosomes with endosomes). The fusion of late endosomes and lysosomes requires *N*-ethylmaleimide sensitive factor (NSF) and soluble NSF attachment proteins (SNAPs). **b** | *trans*-SNARE (SNAP receptor) complex formation requires syntaxin-7, VTI1B (Vps10 tail interactor-1B) and syntaxin-8 in both homotypic late endosome fusions and heterotypic late endosome–lysosome fusions. Whereas vesicle-associated membrane protein-8 (VAMP8) is required for homotypic late endosome fusion, VAMP7 is needed for heterotypic late endosome–lysosome fusions. Two different combinatorial *trans*-SNARE complexes are shown. **c** | The release of lumenal Ca^{2+} (shown only for heterotypic fusion) leads to phospholipid bilayer fusion. Reformation of lysosomes from hybrid organelles requires the loss of mannose-6-phosphate receptors, SNARE retrieval and condensation of lumenal content to produce dense-core lysosomes. It should be noted that all five of the SNAREs shown have been observed on both late endosomes and lysosomes. Release of lumenal Ca^{2+}, which is necessary for membrane fusion between endosomes and lysosomes, is probably promoted by *trans*-SNARE complex formation, as has been described for homotypic vacuole fusion in yeast[133].

Liposome
An artificial lipid vesicle that encloses an aqueous interior.

Isopycnic ultracentrifugation
Centrifugation of samples (organelles or macromolecules) in a density gradient until an equilibrium is reached, such that the density of the sample is the same as that part of the density gradient in which it equilibrates.

Retromer complex
A complex of cytoplasmic proteins that are required for some retrograde membrane-trafficking pathways that deliver cargo from endosomes to the *trans*-Golgi network.

tetanus-neurotoxin-insensitive VAMP (TI-VAMP) or synaptobrevin-like-1 (SYBL1)) for heterotypic late endosome–lysosome fusions[64] (FIG. 3b). As described below and in BOX 2, VAMP7 is found in numerous combinatorial SNARE complexes, several of which involve lysosomes. VAMP7 is an unusual R-SNARE because it has a relatively long (~110 amino acid) N-terminal extension that might function as a regulatory domain. This so-called longin domain is required for the delivery of VAMP7 to late endocytic compartments as a result of binding to AP3 (REF. 65).

Membrane fusion. It has not been proven whether *trans*-SNARE complex formation on its own is sufficient to result in phospholipid bilayer fusion between endosome and lysosome membranes. *Trans*-SNARE complexes can cause fusion of liposomes but kinetic differences with biological fusion reactions have been reported[61].

In the case of heterotypic fusion of endosomes with lysosomes, there is evidence from cell-free content-mixing assays that membrane fusion is dependent on Ca^{2+} and calmodulin[13]. Ca^{2+} is released from the lumen of the fusing organelles late in the mechanistic pathway of fusion (FIG. 3c).

Hybrid organelles. The immediate product of direct and complete fusion between a late endosome and a lysosome is a hybrid organelle that contains a full complement of lysosomal enzymes but still contains some MPRs. This hybrid organelle is the site of degradation of endocytosed macromolecules. The demonstration of direct fusion between the two organelles is consistent with the idea that lysosomes are, fundamentally, storage granules for mature lysosomal enzymes, and that they periodically fuse with late endosomes to form a compartment, sometimes referred to as a 'cell stomach', in which degradation occurs[66]. It is noticeable that lysosomes morphologically resemble regulated secretory granules[66], and their content might well be less aqueous than the cytoplasm or the lumen of the endosome. It is also known that lysosomes behave as dense organelles following isopycnic ultracentrifugation on a variety of density gradients used for subcellular fractionation. Hybrid organelles have an intermediate density between those of lysosomes and late endosomes[48].

Reformation of lysosomes from hybrids. The direct and complete fusion of late endosomes with lysosomes would consume both organelles if no recovery process occurred. Therefore, lysosome reformation from hybrid organelles is necessary and requires content condensation and a membrane-retrieval process to remove endosomal membrane proteins and recycle SNAREs. Lysosomes can be reformed from hybrid organelles in a cell-free system, in which content condensation requires a proton-pumping ATPase and lumenal Ca^{2+} (REF. 13). In a study of asialoglycoprotein endocytosis and degradation in rat hepatocytes, it was also suggested that phosphoinositide-3-kinase activity is required for the reformation of dense lysosomes from hybrid organelles[67]. In live-cell experiments following endosome–lysosome fusion, small vesicular tubular structures have been observed budding off hybrid organelles, consistent with a lysosome reformation process[12]. VAMP7-positive vesicles were also observed budding from terminal endocytic compartments in a live-cell study of organelles containing the Niemann–Pick C1 protein[68].

Overall, the lysosome reformation process will be one of maturation and, by definition, it is only at the point at which no MPRs are detectable in the organelles that they can be called lysosomes. One candidate for the machinery that mediates membrane retrieval from the hybrid organelles is the retromer complex. This complex was first described in yeast as a complex of Vps5, Vps17, Vps26, Vps29 and Vps35. Depletion of the Vps26 orthologue in mammalian cells leads to a phenotype in which there is some swelling and vacuolarization of lysosomal compartments[15,69].

Box 2 | **SNARE complexes that include vesicle-associated membrane protein-7**

At the centre of the parallel four-helix SNAREpin bundle is an ionic layer comprising an arginine (R) and three glutamine (Q) residues, each of which is contributed by a different soluble *N*-ethylmaleimide sensitive factor attachment protein receptor (SNARE) in the *trans*-SNARE complex. These proteins are termed R-SNARE and Qa-, Qb- and Qc-SNAREs, respectively. Intriguingly, the R-SNARE vesicle-associated membrane protein-7 (VAMP7) can form combinatorial *trans*-SNARE complexes with several Q-SNAREs. Each *trans*-SNARE complex must contain one R-SNARE helix and three Q-SNARE helices — one each of Qa, Qb and Qc. The Qb and Qc helices can be contributed by separate SNAREs, but the synaptosome-associated proteins of 25 kDa and of 23 kDa (SNAP25 and SNAP23, respectively) each contain a Qb and Qc motif joined by a flexible linker[59,61]. Possible *trans*-SNARE complexes that include the R-SNARE VAMP7 are listed in the table below, together with their proposed function. All complexes are mammalian unless otherwise stated.

Qa SNARE	Qb SNARE	Qc SNARE	Qb/c SNARE	Membrane fusion and proposed function
Syntaxin-7	VTI1B	Syntaxin-8		Lysosome–late endosome[64].
Syntaxin-7	Vti1	Syntaxin-8		Macropinosome fusion (*Dictyostelium discoideum*)[95].
Syntaxin-4			SNAP23	Lysosome–plasma membrane[77]. Function in membrane repair, neurite outgrowth[130] and granule exocytosis[81,82].
Syntaxin-3			SNAP23	Vesicle–apical plasma membrane[131].
Syntaxin-1			SNAP25	Vesicle–neurite plasma membrane[65,132]. Function in neurite outgrowth.

VTI, VPS10 tail interactor.

Defects in lysosome reformation. It has been suggested that the absence of the lysosomal cation transporter mucolipin-1 (REF. 70) in cells from patients with the autosomal recessive disease mucolipidosis type IV (ML IV), and its orthologue CUP-5 in *Caenorhabditis elegans*, results in a failure to reform lysosomes from hybrids that thereby causes disease[71]. This might occur because intraorganellar Ca^{2+} is required for the condensation of content when lysosomes re-form from hybrid organelles. However, the loss of mucolipin-1 or CUP-5 might also cause defects in the retrieval of components from endosome–lysosome hybrids, possibly by inhibiting the formation of specific transport vesicles or other intermediates. Consistent with this hypothesis, the defective trafficking of lactosylceramide, a neutral glycosphingolipid, from late endocytic organelles to the Golgi complex in ML IV cells can be rescued by the expression of a correctly localized form of mucolipin-1 with an intact cation pore[72].

Lysosome fusion with the plasma membrane

In recent years, it has been recognized that in many cell types, conventional lysosomes might fuse with the plasma membrane in response to an increase in the concentration of cytosolic Ca^{2+} that triggers lysosome exocytosis[73]. Such lysosome exocytosis provides the extra membrane for plasma-membrane wound repair[74] and allows the formation of a parasitophorous vacuole; for example, during invasion of cells by *Trypanosoma cruzi*[75].

Insights into the mechanism. Similar to lysosome fusion with endosomes, fusion with the plasma membrane is also controlled by SNAREs. Fusion of lysosomes with the plasma membrane in NRK cells requires the R-SNARE VAMP7. In this situation, VAMP7 forms a *trans*-SNARE complex with the Q-SNAREs syntaxin-4 and synaptosome-associated protein of 23 kDa (SNAP23)[77]. Syntaxin-4 contains a Qa-SNARE motif and SNAP23 contains both a Qb and a Qc motif joined by a flexible linker (BOX 2). Lysosome exocytosis is regulated by the Ca^{2+} sensor synaptotagmin-VII, which may be required for both temporal and geometric control of the fusion pore that is formed at the cell surface. Synaptotagmin-VII restricts both the kinetics and the extent of Ca^{2+}-dependent fusion[76].

Secretory lysosomes. Some cell types contain specialized lysosomal compartments that store newly synthesized secretory proteins, which are referred to as secretory lysosomes or lysosome-related organelles. These include melanosomes, class II major histocompatibility complex compartments[78], basophil granules, neutrophil azurophil granules, platelet-dense granules, mast-cell secretory granules, eosinophil-specific granules and cytotoxic T lymphocyte lytic granules[79]. In cytotoxic T lymphocytes, the lytic granules are the only lysosome-type organelle present and they serve a dual function: they act as the store of acid hydrolases for the digestion of endocytosed macromolecules, and they contain secretory products, such as perforin, that function at the neutral pH that is encountered when the granules fuse with the plasma membrane. In other cell types (for example, melanocytes), lysosome-related organelles exist alongside conventional lysosomes and their biogenesis is distinct but related to that of lysosomes.

In cytotoxic T lymphocytes, the lytic granules are delivered to the immunological synapse that is formed between the lymphocyte and the target cell. Following recognition of a foreign antigen, the lymphocyte centrosome polarizes towards the target cell and makes contact with the inside of the lymphocyte plasma membrane[80]. The secretory lysosomes are then transported along microtubules towards the centrosome at the immunological synapse (FIG. 4). The machinery that is required

Parasitophorous vacuole
A membrane-bound organelle that contains an intracellular parasite; the membrane that surrounds the organelle is derived from the host cell but is modified by the parasite to facilitate its survival and growth.

Melanosome
A membrane-bound organelle that contains melanin and is formed in melanocytes.

Basophil granule
A granule in white blood cells that stains with basophilic dyes.

Immunological synapse
A specialized contact area between a T lymphocyte and an antigen-presenting cell.

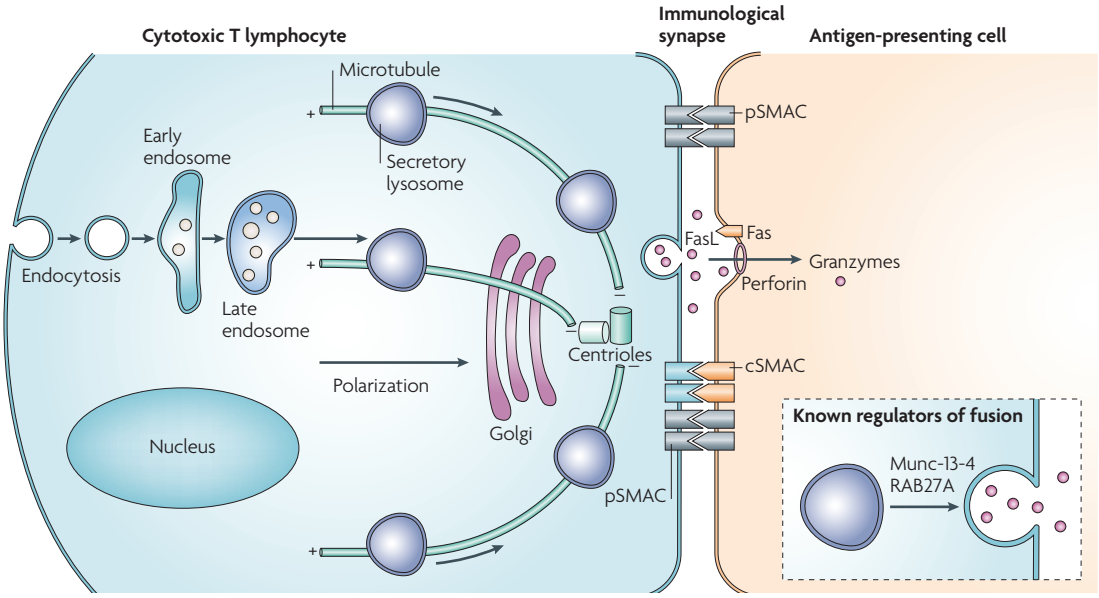

Figure 4 | **Secretory lysosomes in cytotoxic T lymphocytes.** In cytotoxic T lymphocytes, the lysosomes serve a dual purpose: they can degrade endocytosed material and can also fuse with the plasma membrane at the immunological synapse to release their lumenal lytic granule contents. Lysosome fusion occurs when the T-cell receptor recognizes antigen on an antigen-presenting cell. Activation of the T cell causes the centromere to move to the plasma membrane, bringing the secretory lysosomes (which are attached to microtubules) into close proximity with the plasma membrane to aid organelle–plasma-membrane fusion. Known regulators of the fusion process are the small GTPase RAB27A and its effector Munc-13-4. Lysosome fusion occurs next to the central supra-molecular activation complex (cSMAC), where T-cell receptors are involved in target-cell recognition. cSMAC is surrounded by cell-adhesion molecules that form the peripheral (p)SMAC. The lytic granules that induce apoptosis of the antigen-presenting cell contain membrane-bound Fas ligand (FasL), the pore-forming protein perforin and proteases (granzymes).

for the fusion of these lytic granules with the plasma membrane has been more extensively studied than the exocytosis of any other secretory lysosome, not least because of the availability of cells from patients with mutations in the genes required for this process. RAB27A has been implicated in the docking of lytic granules to the plasma membrane, and its effector Munc-13-4 has been implicated in a priming step required for fusion, although the SNARE proteins that are required have not yet been identified. It has been proposed that the lysosome-related organelles of eosinophils and neutrophils require VAMP7 for granule release[81,82] but, in the case of platelets, the study of cells from VAMP8-deficient mice has implicated VAMP8 as a necessary R-SNARE for the fusion of dense granules, alpha granules and lysosomes with the plasma membrane[83]. It is possible that the SNAREs involved may be cell-type specific.

Alpha granule
A platelet granule that contains several growth factors, clotting proteins and the adhesion molecule P-selectin.

Phagosomal cup
A cup-shaped structure, formed principally by invagination of the plasma membrane during the early stages of phagocytic uptake of particles by cells. Membrane that originates from other organelles may be added to it.

Lysosome fusion with phagosomes

Phagocytosis is an essential process by which specialized cells engulf invading pathogens, apoptotic cells and other foreign particles that are >0.5 μm in diameter. This often occurs by a zippering mechanism, in which pseudopods (actin-driven protrusions of the plasma membrane) engulf a target by repeated receptor–ligand interactions. Phagocytosis triggers the activation of multiple transmembrane signalling pathways that lead to the reorganization of the actin cytoskeleton and the formation of a sealed intracellular compartment — the phagosome. It is now widely accepted that the phagosome 'matures' by multiple transient interactions with endosomal compartments, including lysosomes, to form a hybrid-like organelle termed the phagolysosome[84]. The primary function of the phagolysosome is to degrade the phagocytosed particle. Endoplasmic reticulum membrane might also be incorporated into the newly formed phagosome, but this is controversial[85]. It has been suggested that the additional membrane that is required for the formation of phagosomal cups (when there is a high load of phagocytic particles) might be derived from lysosomes that fuse with the plasma membrane by a process that may be similar to the calcium- and synaptotagmin-VII-dependent exocytosis of lysosomes[86]. *In vitro*, phagosomes are capable of fusing with both early and late endosomes and with lysosomes[87,88], as shown by various different assays that demonstrate these capabilities[87–90].

Tethering and docking mechanisms. The complexity of phagosome maturation has made it difficult to dissect out the precise molecular events that lead to phagolysosome biogenesis, but it is known to proceed via tethering and docking steps[89]. Requirements for RAB5 (REF. 87), RAB7 and its effector RILP[91], and filamentous actin[92] have been reported. The tethering step has been shown to require actin polymerization and calmodulin[89]. Cells that are depleted of lysosomal-associated membrane protein-1 (LAMP1) and LAMP2 show

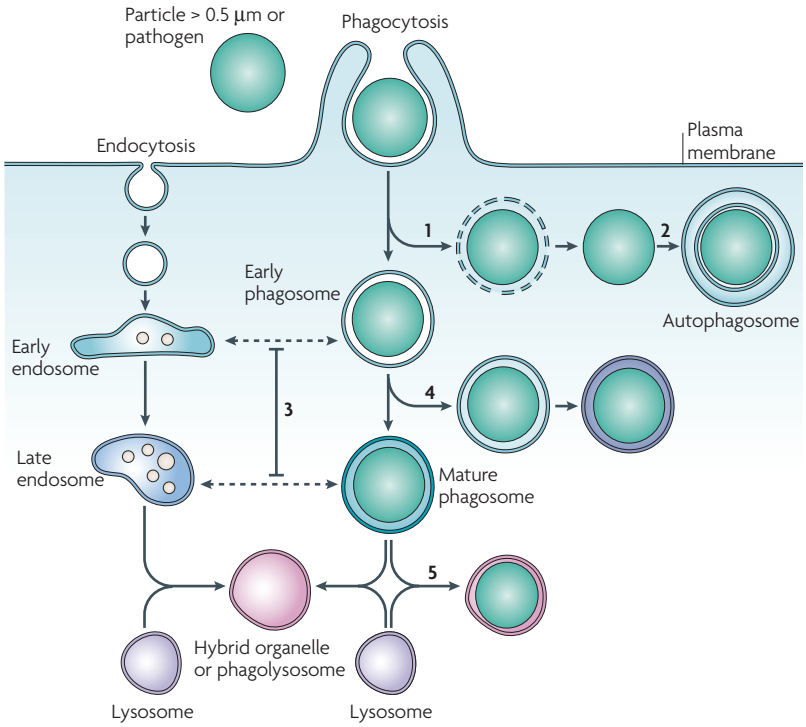

Figure 5 | **Pathogen survival by avoidance of lysosomes.** Phagosome maturation includes multiple interactions with endocytic compartments that eventually give rise to a mature phagosome. This organelle fuses with lysosomes to form an organelle that is similar to a hybrid organelle, namely the phagolysosome, where digestion of the phagocytic particle occurs. Pathogens have evolved multiple strategies to prevent delivery to and/or subsequent degradation at the phagolysosome. These include lysis of the phagosomal membrane and escape into the cytosol (1) with subsequent avoidance of autophagy (2); a delay in phagosomal maturation that aids development of the replicative niche (3); subversion from the phagocytic pathway (4); and survival in the harsh phagolysosomal environment (5).

abnormal phagosome maturation[93]; the phagosomes fail to recruit RAB7 and do not fuse with lysosomes, which is possibly because of impaired movement along microtubules. During normal phagosome–lysosome fusion, some fusion occurs by thin tubular extensions[91] and post-docking events require Ca^{2+} (REF. 89). These features are similar to those that occur during late endosome–lysosome fusion. The identity of the SNARE complex for phagosome–lysosome fusion is unknown, although syntaxin-7 has been implicated[94].

During phagocytosis, macropinosomes are often formed, and some forms of phagocytosis that do not use zippering have been argued to be similar to macropinocytosis[91]. In *Dictyostelium discoideum*, in which macropinosomes fuse with vesicles containing lysosomal enzymes, a *trans*-SNARE complex comprising syntaxin-7, syntaxin-8, Vti1 and Vamp7 has been identified as being required for both homotypic and heterotypic fusion events in the macropinocytic pathway[95].

Lysosome fusion with autophagosomes

Autophagy — or to be more specific, macroautophagy — is an important mechanism for the degradation of cytoplasmic components, including organelles, and has long been known to involve degradation by lysosomal enzymes. It is important in many physiological processes[96], including the response to starvation, cell growth and innate immunity; one example is the removal of pathogenic bacteria when they have been released after endocytosis by non-phagocytic cells[97] (see below).

Mechanisms of fusion. In autophagy, double-membrane vesicles called autophagosomes sequester part of the cytoplasm and then fuse with lysosomes to form hybrid-like organelles called autolysosomes (FIG. 1b). In *S. cerevisiae*, in which the molecular mechanisms of autophagy are best understood, autophagosomes fuse with the vacuole. The mechanism of fusion has similarities with homotypic vacuole fusion, and the SNAREs Vam3 and Vti1 (for which the likely mammalian orthologues are syntaxin-7 and VTI1B, respectively) have both been implicated in the fusion of autophagosomes with the yeast vacuole[98,99]. Members of the HOPS complex have also been shown to be important in autophagosome–vacuole fusion in *S. cerevisiae* and autophagosome–lysosome fusion in *Drosophila melanogaster*[100–102]. Yeast homotypic vacuole fusion and autophagosome–vacuole fusion also require a complex of two proteins, Mon1 (monensin sensitivity-1) and Ccz1 (calcium caffeine zinc sensitivity-1), but the function of these proteins is not clear [103,104]. In mammalian cells, RAB7 has been implicated in the fusion of autophagosomes with lysosomes[105]. Fusion is reduced in cells that are depleted of LAMP1 and LAMP2, although the mechanism behind this effect is not understood[106]. At least some other proteins that are required for autophagosome–lysosome fusion in mammalian cells are likely to be the same as those required for late endosome–lysosome fusion.

Evasion of lysosome fusion by microbes

Several pathogenic microorganisms need to reach either an intracellular compartment or the cytoplasm of a target cell for their survival and replication. However, cellular entry usually requires the use of endocytic and phagocytic pathways that terminate in fusion with lysosomes. Therefore, survival of the microorganism involves avoiding the lysosome or, in the case of *Leishmania* species, surviving the harsh environment of the lysosome itself. Pathogens use several strategies to aid their intracellular survival[107,108] (FIG. 5).

Preventing lysosome fusion. Different mechanisms have evolved by which phagocytosed pathogens can prevent phagolysosome biogenesis. One of the simplest examples is that used by *Escherichia coli* K1, which transcytoses across brain microvascular endothelial cells in an enclosed vacuole that does not fuse with lysosomes[109]. The single bacterial determinant that prevents lysosome fusion is the K1 capsule, which consists of long chains of α-2,8-linked polysialic acid. However, it is unclear how this capsule prevents maturation and fusion with lysosomes when it is present in the vacuole lumen.

Delaying phagolysosome biogenesis. The delay of phagolysosome biogenesis by the pathogens *Salmonella enterica* and *Mycobacterium tuberculosis* has been

Macropinosome
A membrane-bound compartment (organelle), often of ~0.5 μm diameter or larger. It is formed during fluid-phase uptake, particularly in regions of the cell where plasma-membrane ruffling occurs.

α-2,8-linked polysialic acid
A linear homopolymer of *N*-acetyl neuraminic acid monomers that are linked by α-2,8 ketosidic linkages.

extensively studied[110,111]. Both of these organisms produce proteins such as phosphoinositide phosphatases that alter phosphoinositide phosphate concentrations in the phagosome membrane and thereby retard phagosome maturation[112–115]. In the case of phagocytosed *Salmonella enterica* serovar Typhimurium, phagosome maturation is delayed and fusion with lysosomes is prevented as a result of modulating the network of Rab proteins that are recruited to the phagosome[116].

Escaping the phagosome. Some pathogens, including examples from the genera *Listeria*, *Shigella* and *Rickettsia*, rapidly escape their intracellular vacuole by degrading the phagosomal membrane and escaping into the cytosol. *Listeria monocytogenes* has been studied extensively[117] and its escape from the phagosome is mediated, in part, by secretion of the pore-forming toxin listeriolysin O[118]. Listeriolysin O slows the maturation of the phagosomal compartment by causing alterations in both vacuolar pH and Ca^{2+} concentration[119], thereby giving the bacteria time to escape into the cytosol[120]. Escape from a phagosomal compartment into the cytosol is not a fail-safe method for avoiding the lysosome because of the possibility of autophagy, which acts as part of the innate defence system against invading pathogens [97,121]. For example, *Streptococcus pyogenes* is killed by autophagy by lysosomes[97], but it survives and multiplies in autophagy-deficient cells[97]. *M. tuberculosis*, which normally resides in a phagosome, can also be killed by stimulation of the autophagy pathway[122].

Some pathogens can avoid autophagic detection. An example is *L. monocytogenes*, which is only autophagocytosed after release from phagosomes if it is made metabolically inactive by chloramphenicol treatment[123]. *Shigella flexneri* also evades autophagic delivery to the lysosome by a mechanism that is dependent on the secretion of IcsB, which camouflages the bacterium from the autophagic host defence system[124,125]. Other pathogens, including *Legionella pneumophila*, *Coxiella brunetti* and *Brucella abortus*, can reside in an autophagosomal compartment where they multiply, but the precise mechanisms by which they delay progression to an autolysosome are unclear[126].

Conclusions and future directions

There is now compelling evidence that lysosomes can fuse with late endosomes, the plasma membrane, phagosomes and autophagosomes. Kissing events and direct fusion with late endosomes are the means by which endocytosed and newly synthesized macromolecules are delivered to lysosomes. Following fusion, which is achieved after the formation of a specific *trans*-SNARE complex, the resulting hybrid organelle is the site of degradation of endocytosed macromolecules. Lysosomes are re-formed from the hybrid organelle by a maturation process. Although the essential features of lysosome fusion with late endosomes and the plasma membrane have been established, the regulation of these processes, and of lysosome fusion with other organelles, is far from clear. There is a lack of knowledge about the signalling pathways that trigger lysosome fusion with the plasma membrane in response to membrane damage and influx of Ca^{2+}. Little is known about what 'prepares' an endosome, autophagosome or phagosome for fusion with a lysosome. There is also much mechanistic detail to add to what we currently understand about the lysosomal fusion process, in particular with regard to fusion with autophagosomes and phagosomes.

The implications of a greater understanding of lysosome fusion are considerable. For example, it would be of great benefit to have the knowledge to overcome the inability of lysosomes to fuse with vacuoles containing microorganisms that survive and multiply in a protected endocytic niche. By finding better ways of upregulating autophagic pathways and the formation of autophagolysosomes, aggregate-prone cytosolic proteins that cause a range of proteinopathies including Huntington's disease and Parkinson's disease could be removed[127]. The recognition that mature lysosomes should be regarded as storage organelles for degradative enzymes, with the hydrolysis of substrates occurring in hybrid organelles, underlines the importance of understanding how hybrid organelles are formed. This will allow us to achieve a better understanding of lysosome function, whether it be in the conventional tasks of protein, lipid and carbohydrate degradation[128] following endocytosis, autophagy and phagocytosis, or in the recently recognized roles such as necrotic cell death[129] and cell surface repair.

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Competing interests statement
The authors declare no competing financial interests.

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