

Lysosomes as dynamic regulators of cell and organismal homeostasis

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Abstract | Exciting new discoveries have transformed the view of the lysosome from a static organelle dedicated to the disposal and recycling of cellular waste to a highly dynamic structure that mediates the adaptation of cell metabolism to environmental cues. Lysosome-mediated signalling pathways and transcription programmes are able to sense the status of cellular metabolism and control the switch between anabolism and catabolism by regulating lysosomal biogenesis and autophagy. The lysosome also extensively communicates with other cellular structures by exchanging content and information and by establishing membrane contact sites. It is now clear that lysosome positioning is a dynamically regulated process and a crucial determinant of lysosomal function. Finally, growing evidence indicates that the role of lysosomal dysfunction in human diseases goes beyond rare inherited diseases, such as lysosomal storage disorders, to include common neurodegenerative and metabolic diseases, as well as cancer. Together, these discoveries highlight the lysosome as a regulatory hub for cellular and organismal homeostasis, and an attractive therapeutic target for a broad variety of disease conditions.

Membrane contact sites

Membrane domains where organelles are closely (10–30 nm) held together by tethering proteins.

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<https://doi.org/10.1038/s41580-019-0185-4>

Ever since its discovery by Christian de Duve in the 1950s¹, the lysosome has been known as a membrane-enclosed cytoplasmic organelle responsible for the degradation of a variety of biological macromolecules, including proteins, lipids, carbohydrates and nucleic acids^{2,3}. These macromolecules reach the lysosome by various routes, including the endocytic, phagocytic and autophagic pathways, to be degraded in the lysosomal lumen by more than 60 acid hydrolases for subsequent reutilization by the metabolic processes of the cell. In addition to the luminal hydrolases, the lysosome also contains specific sets of integral membrane proteins and lysosome-associated proteins, the latter category being represented by proteins that dynamically interact with the lysosomal surface under certain conditions (FIG. 1).

Each mammalian cell comprises between 50 and 1,000 lysosomes distributed throughout the cytoplasm. Owing to their role in terminal degradation, lysosomes have often been referred to as the 'garbage-disposal system' of the cell. In addition, lysosomes have long been regarded as 'housekeeping' organelles that perform their degradative function irrespective of the cell's status and in relative isolation from other organelles. Moreover, lysosomes have been thought of as static organelles whose cytoplasmic localization does not change over time. Lastly, lysosomal dysfunction has been historically associated with rare diseases such as lysosomal storage disorders (LSDs) caused by impaired degradation of lysosomal substrates. For all of these reasons, the study of lysosomes remained a highly specialized area

of research, with few connections to other aspects of cell and organismal physiology.

This narrow view of the lysosome has been dramatically overturned by several recent discoveries. First, lysosomes have been found to participate in many other cellular processes besides degradation, including metabolic signalling, gene regulation, immunity, plasma membrane repair and cell adhesion and migration. Second, the number, composition and functions of lysosomes have been shown to vary in response to environmental cues, as well as to cellular and organismal needs. Third, lysosomes have been found to engage in physical and functional interactions with other cellular structures, including the formation of membrane contact sites. Fourth, lysosomes have been observed to move around the cytoplasm, often changing their size and shape, or undergoing fusion or fission, as they go. Finally, changes in lysosomal function have been implicated in the pathogenesis of common diseases, including neurodegenerative and metabolic disorders, as well as cancer. In this article, we review these recent developments in lysosome biology that now place the lysosome at the centre of a complex regulatory network for the control of cellular and organismal homeostasis.

The lysosome as a signalling hub

Cellular organelles constantly communicate with each other by either establishing contacts or sending signals. While the core function of an organelle is typically executed in the lumen, signalling occurs at its surface.

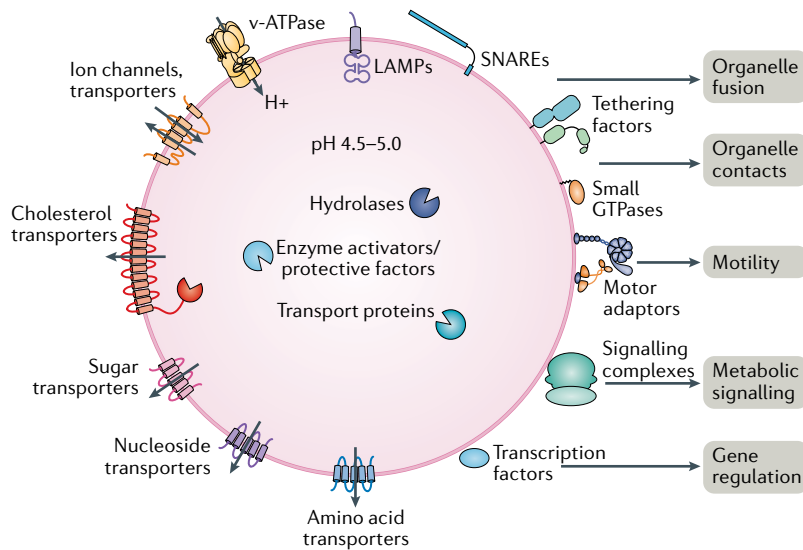


Fig. 1 | Properties of lysosomes. The lysosome comprises a specific set of luminal, integral-membrane and peripherally associated proteins. The lysosomal lumen contains acid hydrolases that degrade different substrates, enzyme activators and protective factors that aid in the degradation, as well as transport factors such as lipid transfer Niemann–Pick type C2 protein (NPC2), which shuttles free cholesterol to NPC1 for export from the lysosome. The acidic pH of the lysosomal lumen is maintained by a vacuolar ATPase (v-ATPase) embedded in the limiting membrane. In addition, the lysosomal membrane comprises highly glycosylated lysosome-associated membrane proteins (LAMPs) that among other functions protect the lysosomal membrane from degradation, ion channels and transporters that maintain ion homeostasis, cholesterol and other lipid transporters, solute carriers that participate in the export of sugars, nucleosides, amino acids and other products of lysosomal degradation, and SNAREs that mediate fusion of lysosomes with other organelles. The cytosolic face of lysosomes serves as a platform for the dynamic association of proteins and protein complexes (shown in the right part of the figure), including the mechanistic target of rapamycin complex 1 and its regulators that transduce nutrient and growth factor signals (FIG. 2), transcription factors such as TFEB and TFE3 that regulate lysosome biogenesis, autophagy and energy metabolism (FIG. 3), tethering factors that promote lysosome fusion or contacts with other organelles (FIGS 4, 5), adaptor or scaffold complexes that couple lysosomes to microtubule motors such as kinesins and dynein–dynactin (FIG. 6) and small GTPases that control the recruitment and activation of all of the aforementioned molecules.

RAG GTPases

RAGA, RAGB, RAGC and RAGD are small GTPases that belong to a subfamily of the RAS-related GTPases. They act as heterodimers in which RAGA or RAGB interacts with RAGC or RAGD. Their activation by amino acids mediates the recruitment of mechanistic target of rapamycin complex 1 to the lysosomal surface.

Regulator

Protein complex composed of five subunits (LAMPOR1–LAMPOR5). It is located on the lysosomal surface, where it interacts with RAG GTPases for the recruitment of mechanistic target of rapamycin complex 1 to the lysosome. It also interacts with BORC for the regulation of lysosome positioning.

Recent studies revealed that several organelles, including mitochondria⁴, melanosomes⁵, peroxisomes⁶ and lysosomes⁷, act as ‘launch pads’ for signals to other cellular compartments, including the nucleus⁸. Here we will focus on specific types of lysosomal signalling pathways that respond to different environmental stimuli and play critical roles in the regulation of various functions, ultimately allowing maintenance of cellular homeostasis.

Lysosomal nutrient sensing and mTORC1 signalling.

Being the main mediator of cellular catabolism, the lysosome is in a unique position to use information on cellular degradation and recycling processes as a proxy to sense the cell’s nutritional status. The mechanisms that underlie lysosomal nutrient sensing have recently become a hot topic in cell biology. A major advance in this field was the discovery that nutrient-regulated mechanistic target of rapamycin complex 1 (mTORC1), a key regulator of cellular biosynthetic pathways⁹, dynamically associates with the lysosome under specific conditions⁷ (FIG. 2). A key function of mTORC1 is to support cell anabolism and growth in the presence of nutrients and

growth factors, while inhibiting catabolic pathways, such as autophagy through the phosphorylation of Unc-51-like kinase 1 (ULK1)¹⁰. Of note, mTORC1 also regulates lysosome re-formation during autophagy, a process that helps to restore a full complement of functional lysosomes during prolonged starvation¹¹.

Activation of mTORC1 requires its dynamic recruitment to the lysosomal surface, which is mediated by the amino acid-dependent activation of heterodimeric RAG GTPases and their interaction with Regulator^{7,12–17}. The RAG-dependent recruitment of mTORC1 to the lysosomal surface can also be induced by cholesterol through the involvement of cholesterol-binding Niemann–Pick type C1 protein (NPC1)¹⁸. In addition to mTORC1, RAG GTPases modulate the lysosomal recruitment of other nutrient-responsive molecules, including the mTORC1 regulators tuberous sclerosis complex (TSC)¹⁹ and folliculin (FLCN)–folliculin-interacting protein 1 (FNIP) complexes²⁰, as well as TFEB²¹, a master modulator of lysosome biogenesis and autophagy⁸ (see also the section Lysosomal adaptation) (FIG. 2). Additional details on lysosomal nutrient sensing and on the regulation and functions of mTORC1 at the lysosomal membrane can be found in other recent reviews^{9,17}.

Lysosomal Ca^{2+} signalling. Lysosomal Ca^{2+} is key for various lysosomal functions. Release of Ca^{2+} is required for the fusion of lysosomes with other cellular structures, including endosomes, autophagosomes and the plasma membrane^{22,23}, thereby regulating endocytic membrane trafficking, autophagy and repair of membrane damage (see also the section Interactions with other organelles). Furthermore, lysosomal Ca^{2+} release is involved in the formation of contact sites with the endoplasmic reticulum (ER), which in turn is able to refill the lysosome with Ca^{2+} (REF.²⁴). Ca^{2+} homeostasis is also important for lysosomal acidification, a requirement for the activity of lysosomal hydrolases²⁵. Three main types of Ca^{2+} channels have been identified in the lysosomal membrane of mammalian cells: transient receptor potential cation channels of the mucolipin family (TRPML), two-pore channels (TPC) and the trimeric Ca^{2+} two-transmembrane channel P2X₄ (REFS^{22,23}). Some of these channels are found exclusively on endolysosomes, whereas others have additional locations.

Lysosomal Ca^{2+} channels respond to a variety of stimuli, such as pH, nutrients and cellular stress, as well as to small molecules such as ATP, nicotinic adenine dinucleotide phosphate, phospholipids and sphingosine, suggesting that their activities can be differentially modulated depending on cell conditions, thus allowing more selective Ca^{2+} signalling responses that are tailored to the needs of the cell.

Perhaps the best characterized lysosomal Ca^{2+} channel is TRPML1, also known as mucolipin 1 (REF.²⁶). The gene encoding TRPML1 is mutated in an LSD named mucopolidosis type IV, which is characterized by early onset and progressive neurodegeneration^{27,28}. TRPML1 activity mediates Ca^{2+} release from the lysosomal lumen to the cytosol and can be activated by several stimuli, including starvation^{29,30} and reactive oxygen species³¹. TRPML1 is also activated by a specific phosphoinositide,

phosphatidylinositol 3,5-bisphosphate, which links lysosomal Ca^{2+} signalling to intracellular trafficking processes³².

FIGURE 2 shows the main lysosomal processes that are regulated by TRPML1-mediated Ca^{2+} release:

lysosomal exocytosis and plasma membrane repair³³, autophagosome–lysosome fusion²⁶, endosome–lysosome fusion, lysosome size³⁴ and lysosome re-formation from hybrid organelles following fusion³⁵. TRPML1 is also involved in a positive-feedback loop with TFEB,

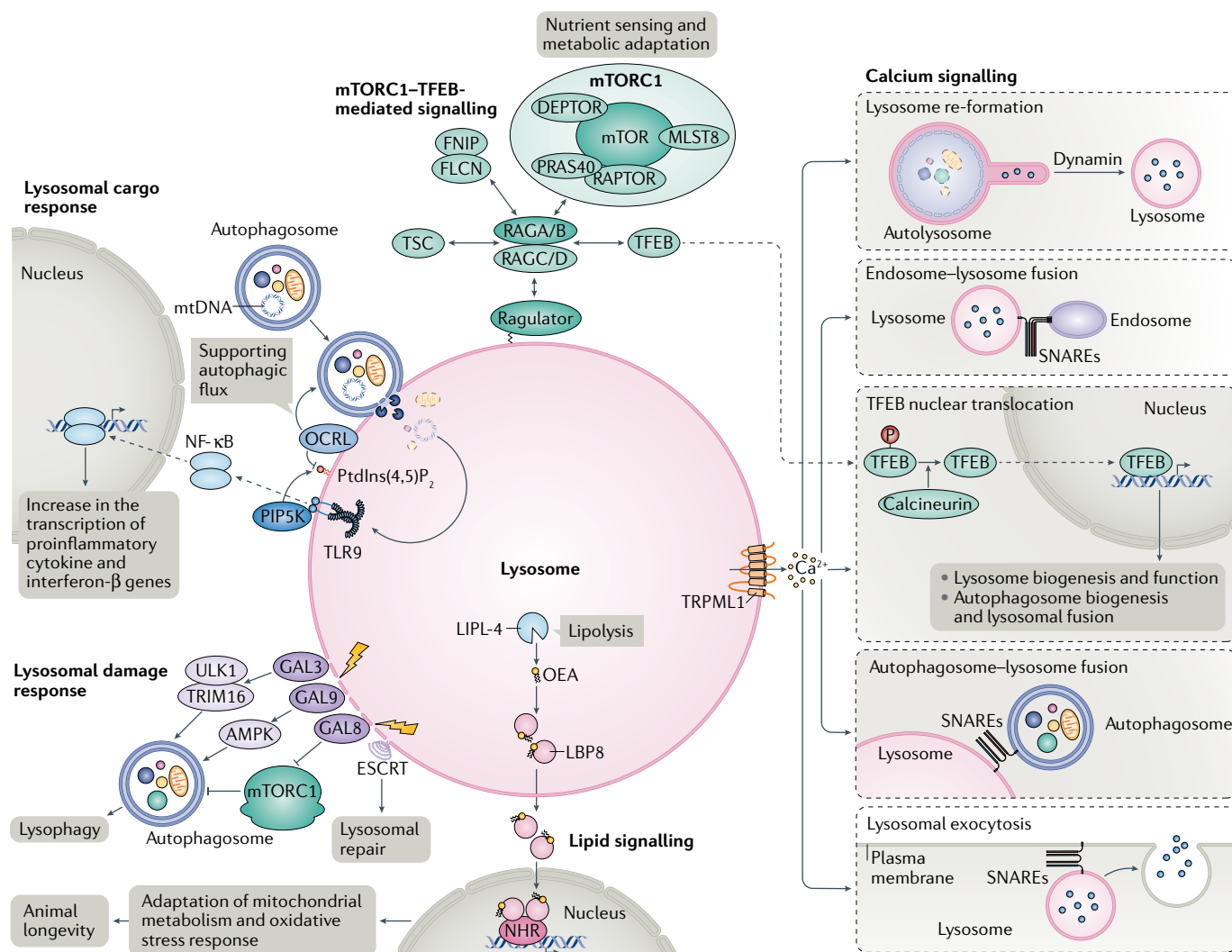


Fig. 2 | The lysosome as a signalling hub. Multiple cellular processes are regulated by signalling pathways initiated from the lysosomal surface. RAG GTPases mediate the dynamic association of several nutrient-modulated protein complexes to the lysosomal surface, including the key regulator of lysosomal function TFEB (FIG. 3), as well as the nutrient sensor and growth regulator mechanistic target of rapamycin complex 1 (mTORC1), together with its regulators tuberous sclerosis complex (TSC), and folliculin (FLCN)–folliculin-interacting protein (FNIP). Ca^{2+} release from lysosomes modulates the induction of several cellular processes, including lysosomal re-formation from hybrid organelles, endosome–lysosome fusion, TFEB nuclear translocation, autophagosome–lysosome fusion and lysosomal exocytosis. In addition, at least in *Caenorhabditis elegans*, the lysosomal lipase LIPL-4 generates the lipid oleoylethanolamide (OEA), which binds to lipid-binding protein 8 (LBP8). The OEA–LBP8 complex is exported from the lysosome and translocated into the nucleus for the activation of the nuclear hormone receptors NHR49 and NHR80, which drive genes responsible for the adaptation of mitochondrial metabolism and oxidative stress response, thereby contributing to animal longevity. Lysosomal damage triggers the recruitment of galectins (GAL3, GAL8 and GAL9), which regulate lysosomal removal through autophagy (lysophagy) via the modulation of mTORC1, 5'-AMP-activated protein kinase (AMPK), and Unc-51-like kinase 1

(ULK1)–tripartite motif-containing protein 16 (TRIM16). Small disruptions of the lysosomal membrane activate a lysosomal repair mechanism that is dependent on the recruitment of the endosomal sorting complex required for transport (ESCRT) machinery that allows membrane sealing. The lysosome is also able to sense the incoming autophagic cargo via the recognition of mitochondrial DNA (mtDNA) by Toll-like receptor 9 (TLR9), which is recruited to lysosomes on autophagy induction. This mediates a 'lysosomal cargo response' that involves TLR9 activation and consequent recruitment of phosphatidylinositol 4-phosphate 5-kinases (PIP5K) and generation of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂), which mediates the recycling of SNAREs involved in autophagosome–lysosome fusion. However, to maintain autophagic flux, PtdIns(4,5)P₂ generation needs to be controlled in time and space, as PtdIns(4,5)P₂ inhibits the lysosomal Ca^{2+} channel TRPML1, which is required for autophagosome–lysosome fusion events. This control over PtdIns(4,5)P₂ levels is provided by the inositol polyphosphate 5-phosphatase activity of Lowe oculocerebrorenal syndrome protein (OCRL). In addition to the regulation of autophagic flux, TLR9-mediated signalling also governs the activation and nuclear translocation of the transcription factor nuclear factor-κB (NF-κB) and an increase in the transcription of proinflammatory cytokine and interferon-β genes, which when uncontrolled can lead to chronic inflammation.

target for pharmacological modulation in a variety of diseases.

Lysosome-dependent cell death and endolysosomal damage response. Several conditions, such as infections and hyperuricaemia, or treatment with lysosomotropic drugs may damage the lysosome by inducing lysosomal membrane permeabilization or rupture. This damage eventually results in leakage of cathepsins, often leading to a form of programmed cell death known as lysosome-dependent cell death^{39–41}. Lysosome-dependent cell death can take the form of apoptosis, whereby

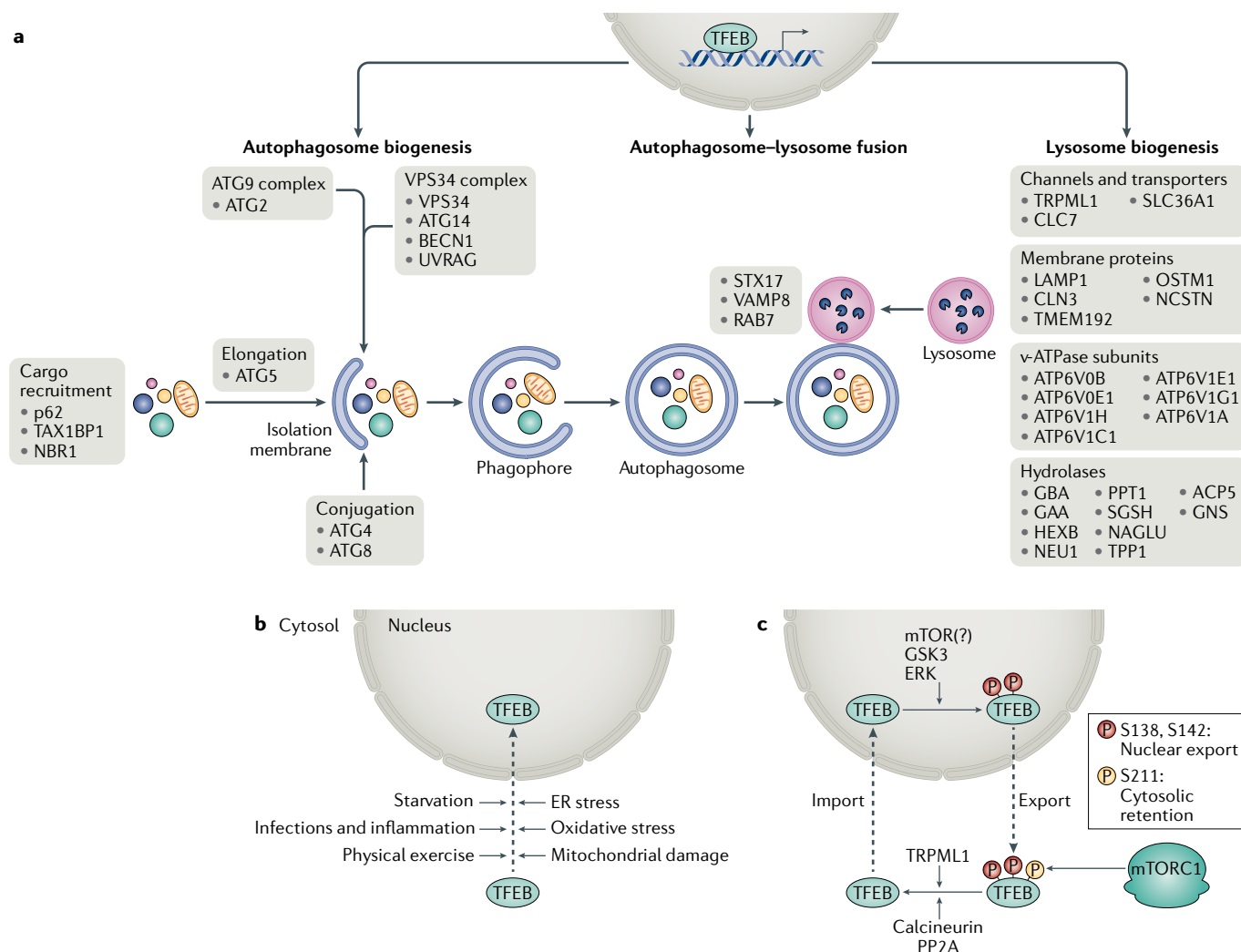


Fig. 3 | The role of TFEB in the transcriptional regulation of the lysosomal-autophagic pathway. **a** | TFEB regulates genes involved in several steps of the lysosome-autophagic pathway, including formation of the isolation membrane, conjugation of ubiquitin-like molecules of the Atg8 family (for example, LC3) and elongation of the autophagosomal membrane, cargo recruitment, autophagosome-lysosome fusion, lysosome-mediated degradation and lysosomal biogenesis. For each process, TFEB direct transcriptional targets, identified by chromatin immunoprecipitation-sequencing analyses, are shown in grey boxes (REF.⁶⁴ and A.B., unpublished observations). **b** | TFEB nuclear translocation is promoted by multiple environmental stimuli, including starvation, bacterial infections, proinflammatory agents, physical exercise, endoplasmic reticulum (ER) stress, oxidative stress and mitochondrial damage, which allow adaptation of the lysosomal-autophagic pathway to cellular needs. **c** | TFEB nucleocytoplasmic shuttling is modulated

by its phosphorylation status. Activation of the TRPML1 Ca^{2+} channel and of the phosphatases calcineurin and protein phosphatase 2A (PP2A) results in TFEB dephosphorylation and nuclear translocation^{29,88}. TFEB phosphorylation on serine S142 and S138 residues — which are mechanistic target of rapamycin (mTOR)-dependent phosphorylation sites — promotes its binding to the exportin CRM1 and subsequent nuclear export (REF.⁹⁷ and A.B., unpublished observations). Whether the nuclear phosphorylation of TFEB is directly or indirectly mediated by mTOR is currently unknown (the former case would necessitate nuclear presence of mTOR). ERK and glycogen synthase kinase 3 (GSK3) have also been proposed as putative kinases for S142/S138 phosphorylation^{62,98,109}. On nuclear export, mechanistic target of rapamycin complex 1 (mTORC1)-mediated phosphorylation of TFEB at S211 induces its binding to 14-3-3 proteins and cytosolic retention^{92–94}. v-ATPase, vacuolar ATPase.

Folluculin

(FLCN). Tumour-suppressor protein that exists as part of a complex with the folluculin-interacting protein and functions as a GTPase-activating protein for the RAGC and RAGD GTPases.

Endolysosomes

Generic term for various types of endosomes and lysosomes.

Sphingosine

Long-chain unsaturated amino alcohol that forms the backbone of a class of membrane lipids known as sphingolipids.

Phosphoinositide

A class of phospholipids comprising a *myo*-inositol head group linked by a glycerol moiety to two fatty acyl chains. Phosphoinositides are minor components of cellular membranes involved in signalling and regulation of membrane dynamics.

14-3-3 proteins

Family of proteins that bind phosphorylated serine or threonine residues on various regulatory proteins such as kinases, phosphatases, transcription factors and signal-transduction proteins.

Hyperuricaemia

Elevated levels of uric acid in blood.

Lysosomotropic drugs

Drugs that concentrate within the acidic lumen of lysosomes and modify lysosomal function.

Pyroptosis

A type of programmed cell death most often triggered by infection with intracellular pathogens and mediated by pore-forming gasdermin D, which permeabilizes the plasma membrane.

Ferroptosis

Iron-dependent form of cell death triggered by inactivation of cellular glutathione-dependent antioxidant defences with consequent accumulation of lipid reactive oxygen species.

Necroptosis

A programmed form of necrosis that is downstream of cell-death receptor signalling, which is generally induced by cell damage but can also be promoted by lysosomal membrane permeabilization.

cathepsins proteolytically activate the proapoptotic proteins BID and BAX, leading to caspase activation^{39,40}, or other forms of cell death such as pyroptosis⁴², ferroptosis⁴³ and necroptosis⁴⁴.

Lysosomal damage can also trigger a process known as the endolysosomal damage response⁴⁵, whereby damaged lysosomes are either eliminated or repaired, to prevent the activation of cell death pathways⁴⁵ (FIG. 2). Elimination and recycling of damaged lysosomes occurs through a selective autophagy pathway termed 'lysophagy'⁴⁶, which is mediated by members of the galectin protein family acting as lysosomal damage sensors. Galectins mediate lysophagy by several mechanisms, including binding of galectin 3 to TRIM16 (REF.⁴⁷), a member of the tripartite motif (TRIM) protein family⁴⁸, as well as binding of galectin 8 to the autophagy receptor NDP52 (REF.⁴⁹). Galectins can also regulate autophagy by both galectin 8-mediated inhibition of mTORC1 and galectin 9-mediated activation of AMPK — a key sensor of reduced cellular energy⁵⁰. One of the consequences of mTORC1 inhibition caused by lysosomal damage is the activation of TFEB, which induces lysosomal biogenesis and autophagy, further facilitating the recycling of damaged lysosomes and their replacement by newly generated ones⁴⁵ (see the section Lysosomal adaptation). Recent studies showed that whereas severe lysosomal damage induces galectin-mediated lysophagy, small disruptions instead activate a lysosomal repair mechanism that is independent of lysophagy and is mediated by the Ca²⁺-dependent recruitment to lysosomal membranes of the endosomal sorting complex required for transport (ESCRT) machinery that seals the damage^{51,52}.

Autophagic cargo sensing and proinflammatory response. Lysosomes sense not only nutrients and metabolites, but also nucleic acids. This task is achieved through members of the Toll-like receptor (TLR) family, which are highly, although not exclusively, expressed in macrophages and dendritic cells^{53,54}. TLRs are able to recognize pathogen-associated molecular patterns, and have a pivotal role in the innate immune response. Of the 13 members of the TLR family, three of them (TLR3, TLR7/8 and TLR9) signal from endolysosomes. These receptors are activated by microbial nucleic acids and, through signal transduction cascades that include different kinds of adaptors, induce the nuclear translocation of either nuclear factor- κ B or interferon regulatory factors to stimulate the production of inflammatory cytokines and/or interferons. The endolysosomal site of action of the aforementioned TLRs restricts their function or site of activation to a confined environment; here, the encounter with exogenous nucleic acids is facilitated, because endocytosis is one of the common mechanisms of pathogen entry into host cells⁵⁵.

TLR9 also has a driving role in the lysosomal response to mitochondrial DNA, which bears similarity to bacterial DNA and can be delivered to the lysosome in the process of autophagy (FIG. 2). This lysosomal cargo response includes local remodelling of phosphoinositides with the production of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂), followed by the recruitment of the endocytic adaptor AP2 and clathrin, which mediate recycling

of SNAREs, such as STX17, used for autophagosome-lysosome fusion. However, PtdIns(4,5)P₂ also inhibits the activity of the lysosomal Ca²⁺ channel TRPML1, which is required for autophagosome-lysosome fusion (FIG. 2). Thus, to sustain the autophagic flux, PtdIns(4,5)P₂ levels need to be controlled in time and space. Such control is governed by the inositol polyphosphate 5-phosphatase activity of Lowe oculocerebrorenal syndrome protein (OCRL)⁵⁶. Overall, this mechanism allows the sensing of the arrival of autophagic cargo at the lysosome to adapt the degradative potential of the cell on autophagy induction.

Apart from supporting autophagic flux, following recognition of mitochondrial DNA, TLR9 also induces nuclear translocation of nuclear factor- κ B and subsequent production of proinflammatory cytokines. In steady-state conditions, this is a self-limiting response; however, this response may be sustained under other conditions that impair the degradative capacity of the lysosome, such as LSDs, leading to a pathological inflammation.

Prolongevity lipid signalling from the lysosome. The lysosome has a critical role in the control of lipid homeostasis and in lipid-mediated signalling⁵⁷. One important lipid family integrated into lysosome biology is the phosphoinositides, which regulate various aspects of lysosome dynamics and function, including its positioning, biogenesis, fusion with autophagosomes and function in lipid transfer at membrane contact sites (recently reviewed elsewhere⁵⁸). Recent studies performed in *Caenorhabditis elegans* also revealed a role for the lysosome in longevity signalling mediated by the lipid oleoylethanolamide (OEA) and the lipid-binding protein LBP8 (REF.⁵⁹) (FIG. 2). OEA is generated by lysosomal lipolysis through the activity of lipase LIPL-4, the orthologue of human LIPA. The OEA-LBP8 complex is subsequently exported to the cytosol and translocates to the nucleus to activate the nuclear hormone receptors NHR49 and NHR80 (REF.⁵⁹). These receptors then induce a transcription programme that regulates mitochondrial β -oxidation and oxidative stress tolerance. This cooperation between lysosomes and mitochondria promotes longevity⁶⁰. At present, it is unclear whether this pathway also operates to promote longevity in mammals⁵⁸.

Lysosomal adaptation

Cellular energy metabolism is influenced by environmental cues. Thus, the lysosome should be able to adapt its function in response to diverse environmental conditions to maintain homeostasis. The question that arises from such hypothesis is how the cell modulates the function of an entire organelle, which is made of hundreds of different proteins. An *in silico*-based approach led to the identification of a transcriptional gene network named 'CLEAR' (for 'coordinated lysosomal expression and regulation'), which includes genes involved in different aspects of lysosomal function and autophagy. This was followed by the discovery of TFEB as the master regulator of this transcriptional network. TFEB and the CLEAR gene network allow lysosomal function and autophagy to be globally controlled^{161,62}.

Galectin protein family
Endogenous carbohydrate-binding proteins (lectins) with specificity for β -galactoside sugars.

Tripartite motif (TRIM) protein family
Family of E3 ubiquitin ligases having RING, B-box and coiled-coil domains.

AMPK
AMP-activated protein kinase that functions as a master regulator of cellular energy metabolism.

ESCRT machinery
Ensemble of complexes known as endosomal sorting complexes required for transport that perform various membrane bending and scission reactions away from the cytoplasm.

Toll-like receptor (TLR) family
Family of transmembrane receptors that recognize pathogen-associated molecular patterns as part of the innate immune response.

Pathogen-associated molecular patterns
Microbial molecules that trigger innate immune responses.

Nuclear factor- κ B
Family of transcription factors that regulate the expression of genes involved in both innate and adaptive immunity.

Interferon regulatory factors
Transcription factors that control both innate and adaptive immunity against invading pathogens.

SNAREs
Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors, which orchestrate membrane fusion events in the cytoplasm.

Oleylethanolamide (OEA). Monounsaturated fatty acid ethanolamide that functions as a bioactive lipid in many physiological processes.

Nuclear hormone receptors
Ligand-activated receptors that bind to specific DNA sequences and regulate gene transcription.

β -oxidation
Catabolic process by which fatty acids are broken down to generate acetyl-CoA in mitochondria.

TFEB and the CLEAR gene network. TFEB belongs to the MiT-TFE family of helix–loop–helix leucine zipper transcription factors together with MITF, TFE3 and TFEC⁶³. TFEB exerts broad control on autophagy and lysosomal function by regulating genes involved in multiple steps of autophagosome biogenesis⁶², autophagosome–lysosome fusion^{62,64} and lysosomal degradation pathways^{61,64}, including lysosomal acidification^{61,64}, lysosomal Ca^{2+} homeostasis^{36,64}, lysosome exocytosis³⁶ and lysosome positioning⁶⁵ (FIG. 3a). Thus, TFEB behaves as a master regulator of autophagic flux by controlling both cargo delivery and substrate degradation⁶². Remarkably, TFEB overexpression also increases the number of lysosomes by promoting the biogenesis of new lysosomes. This aspect may have a crucial role in a disease context in which old lysosomes may be dysfunctional due to accumulation of undegraded substrates^{61,64}.

Notably, other members of the MiT-TFE family, such as TFE3, also regulate lysosome biogenesis and autophagy⁶⁶, and there is evidence for cooperation and partial redundancy between TFEB and TFE3 in the context of metabolic regulation⁶⁷. Overexpression of TFEB, and in some cases TFE3, promoted intracellular clearance and rescued phenotypic abnormalities in a variety of cellular and mouse models of diseases associated with the accumulation of autophagic/lysosomal substrates, such as several types of LSDs^{36,68,69}, common neurodegenerative diseases^{70–73} and metabolic disorders^{74–76}. These observations suggest that manipulation of the MiT-TFE-driven CLEAR network may have broad therapeutic applications.

Furthermore, both TFEB and TFE3 regulate other important cellular processes, such as the unfolded protein response⁷⁷, endocytosis⁷⁸, stem cell differentiation^{79–81} and endosomal recycling through the retromer complex⁸². A recent study showed that TFEB and TFE3 also have roles in the regulation of the circadian rhythm, thus

linking nutrient-driven and circadian clock-driven regulation of gene expression⁸³. TABLE 1 lists additional, tissue-specific, functions of TFEB that were identified by *in vivo* gain-of-function and loss-of-function studies.

Regulation of TFEB by environmental cues. Early studies showed that under normal, steady-state conditions (non-stressed cells under standard culture medium conditions), TFEB is located predominantly in the cytoplasm⁶¹, and that its subcellular localization is dependent on nutrient availability, with TFEB translocating to the nucleus on starvation⁶². A number of conditions were subsequently found to affect TFEB nuclear translocation, including infections⁸⁴, phagocytosis⁸⁵, inflammation⁸⁶, physical exercise⁸⁷, ER stress⁷⁷, oxidative stress⁸⁸ and mitochondrial damage⁸⁹ (FIG. 3b). The main mechanism that regulates the subcellular localization of TFEB, as well as that of the other MiT-TFE factors, is the phosphorylation status of specific serine residues^{62,90} (FIG. 3c). The lysosome-associated, nutrient-sensitive mTORC1 kinase has a major role in the phosphorylation of MiT-TFE factors^{91–95}. Notably, TFEB behaves as an atypical mTOR substrate as it binds to RAG GTPases²¹ and its phosphorylation is resistant to treatment with the mTOR inhibitor rapamycin⁹⁴.

Whereas mTORC1-mediated phosphorylation inhibits the nuclear translocation of MiT-TFE factors, in a feedback response, MiT-TFE factors promote mTORC1 lysosomal recruitment and activity⁹⁶. This effect is mediated by the transcriptional induction of RAGD and RAGC to activate mTORC1, thereby enhancing suppression of the CLEAR pathway and favouring anabolism over catabolism⁹⁶. This mTORC1–TFEB feedback loop has an important role in organismal adaptation to different metabolic conditions, as shown by the impaired ability to reactivate mTOR and protein synthesis in response to leucine after starvation or physical exercise in mice

Table 1 | Tissue/cell-type-specific functions of TFEB

Tissue (cell type)	Function	Loss of function	Refs
Bone (osteoclasts)	Bone resorption	Increased bone mass	229
Liver (hepatocytes)	Lipid catabolism	Enhanced high-fat diet-induced obesity	67,75
Immune system (macrophages, microglia, dendritic cells)	Cytokine production and secretion, phagocytosis, macrophage polarization, migration of dendritic cells, defence against pathogens	Reduced inflammatory response and antimicrobial response, increased susceptibility to infections	84–86,230
Muscle	Glucose homeostasis, mitochondrial biogenesis	Mitochondrial abnormalities, hypoglycaemia, reduced ATP production, intolerance to physical exercise	87
Vascular system	Regulation of vascular development, postischaemic angiogenesis and endothelial cell proliferation	Defects in vascular development and maturation, impaired vessel sprouting, reduced endothelial cell proliferation	231,232
Adipose tissue	Induction of mitochondrial biogenesis in white adipose tissue	Defective browning of white adipose tissue	233
Intestine	Restoration of intestinal epithelial barrier, assembly of Paneth cell secretory granules	Defects in Paneth cell granules, impaired defence against injury, colitis	234
Glia (oligodendrocytes)	Repression of myelination	Induction of remyelination, ectopic myelination	235

MiT-TFE family

Family of helix–loop–helix leucine zipper transcription factors that regulate expression of genes involved in the biogenesis and function of lysosomes and autophagosomes.

Unfolded protein response

Cellular stress response triggered by accumulation of unfolded proteins in the endoplasmic reticulum.

Spermidine

Organic polycation that regulates various cellular processes, including translation.

Vacuolar ATPase

(v-ATPase). Multisubunit, ATP-driven proton pump responsible for the acidification of intracellular compartments.

Tethering complex

Protein complex that promotes SNARE-dependent fusion of membrane-bound organelles.

lacking TFEB in liver and muscle, respectively. This metabolic adaptation mechanism is hijacked by cancer cells to support their demanding metabolic needs⁹⁶ (see also the section Lysosome dysfunction in disease). Overall, this relationship between MiT-TFE factors and mTORC1 links global control of lysosome biogenesis to lysosomal nutrient sensing and explains how lysosomal function can adapt to environmental cues.

In addition to mTOR, other kinases phosphorylate MiT-TFE factors and may affect their subcellular localization, highlighting a complex relationship between MiT-TFE phosphorylation and subcellular localization⁹⁰. Another important player in the regulation of TFEB nuclear translocation is the Ca^{2+} -dependent phosphatase calcineurin. During starvation, calcineurin is activated by lysosomal Ca^{2+} release through the Ca^{2+} channel TRPML1, leading to TFEB dephosphorylation and nuclear translocation²⁹ (FIG. 2). TFEB and TFE3 were also shown to be dephosphorylated by protein phosphatase 2A⁸⁸.

More recently, it was shown that TFEB continuously shuttles between the cytoplasm and the nucleus, and that its overall localization is the result of the net rates of nuclear import and export⁹⁷. TFEB nuclear export is mediated by the major exportin CRM1, which binds to a TFEB nuclear export sequence^{97–99}. TFEB nuclear export is strictly dependent on the phosphorylation of specific serine residues and is blocked by mTOR inhibition, indicating that mTOR promotes TFEB cytoplasmic localization not only by inhibiting its nuclear import but also by inducing its export⁹⁷ (FIG. 3c). The picture that emerges from these studies is that of a signalling mechanism⁹⁴ in which the nucleocytoplasmic shuttling of TFEB, as well as other MiT-TFE transcription factors, conveys information on the lysosomal status to the nucleus.

In addition to phosphorylation-mediated nucleocytoplasmic shuttling, TFEB is also regulated at the level of protein stability. The chaperone-dependent E3 ubiquitin ligase STUB1 is involved in the preferential targeting of phosphorylated TFEB for degradation by the ubiquitin–proteasome pathway¹⁰⁰. Finally, a recent study presented evidence of translational regulation of TFEB through spermidine-mediated induction of the translation factor eIF5A. This mechanism was shown to play an important role in the rejuvenation of old human B cells¹⁰¹.

Other transcriptional regulators of lysosome biogenesis.

Additional factors were found to regulate lysosomal biogenesis and autophagy. The bromodomain protein BRD4 and the methyltransferase G9a act as repressors of the transcription of lysosomal and autophagy genes¹⁰². This pathway appears to be independent of MiT-TFE factors and is induced by starvation through a signalling cascade that involves AMPK and the histone deacetylase SIRT1. The mechanism by which nutrient depletion is relayed to these regulators is still unclear.

STAT3, a member of the STAT family of transcription factors, is also able to regulate lysosomal function. In particular, in the context of mammary gland involution, STAT3 was shown to have a role in the regulation of cathepsins and of lysosome-dependent cell death¹⁰³. Recent studies showed that STAT3 activity is induced

by lysosomal substrate overload in conditions associated with deficiencies of lysosomal proteases, resulting in increased expression of their corresponding genes. This mechanism operates in normally fed cells, and thus is independent of the starvation-induced MiT-TFE pathway¹⁰⁴. Independently of its function in the nucleus, STAT3 was also found to enhance the activity of the vacuolar ATPase (v-ATPase) at the lysosome membrane, thereby increasing lysosomal acidification¹⁰⁵. This leads to inactivation of STAT3 transcriptional activity and further recruitment of STAT3 to the lysosome. Therefore, STAT3 appears to regulate lysosomal function both through transcription regulation of lysosomal genes in the nucleus and through a transcription-independent regulation of the v-ATPase at the lysosomal membrane¹⁰⁵.

Furthermore, AMPK-regulated protein coactivator-associated arginine methyltransferase 1 (CARM1) was shown to act as a transcription coactivator of TFEB in the regulation of lysosomal and autophagic genes¹⁰⁶. Finally, the zinc-finger transcription factor ZKSCAN3 and the helix–loop–helix leucine zipper oncoprotein MYC were reported to act as suppressors of lysosomal biogenesis and autophagy by repressing the expression of autophagic and lysosomal genes^{107–109}. The MYC inhibitory effect is mediated by competition of MYC with TFEB and TFE3 for the binding to lysosomal and autophagic gene promoters. Inhibition of histone deacetylase activity abolishes MYC promoter binding and allows TFEB and TFE3 to activate lysosomal autophagic gene expression¹⁰⁸.

Interactions with other organelles

Although lysosomes were originally thought to exist in relative isolation within the cytoplasm, in recent years they have been found to engage in interactions with other organelles. Some of these interactions lead to fusion, while others involve non-fusogenic contacts with neighbouring organelles (FIG. 4a).

Lysosome fusion with other organelles. All lysosome fusion events — including those with other lysosomes (homotypic fusion) as well as late endosomes, autophagosomes, phagosomes, macropinosomes and the plasma membrane (heterotypic fusion) — are mediated by the assembly of a *trans*-SNARE complex composed of one R-SNARE and two or three Q-SNAREs (FIG. 4b), and are promoted by the release of Ca^{2+} from the lumen of the lysosome³ (FIG. 4c). Lysosome fusion with each organelle type depends on a specific *trans*-SNARE complex and a different set of regulators (FIG. 4b,c). A well-studied fusion event is the merger of lysosomes with late endosomes, the last step in the itinerary of endocytic cargos destined for lysosomal degradation³. Regulators of this particular fusion event include the small GTPase ARL8 and its effector, the heterohexameric tethering complex HOPS, which orchestrate the assembly of the *trans*-SNARE complex¹¹⁰ (FIG. 4c). ARL8 and another small GTPase, RAB7, additionally interact with the tethering protein PLEKHM1 to promote lysosome–late endosome fusion^{111,112} (FIG. 4c). Lysosome–lysosome fusion likely relies on the same machinery, except that the R-SNARE VAMP8 replaces VAMP7 in the process¹¹³.

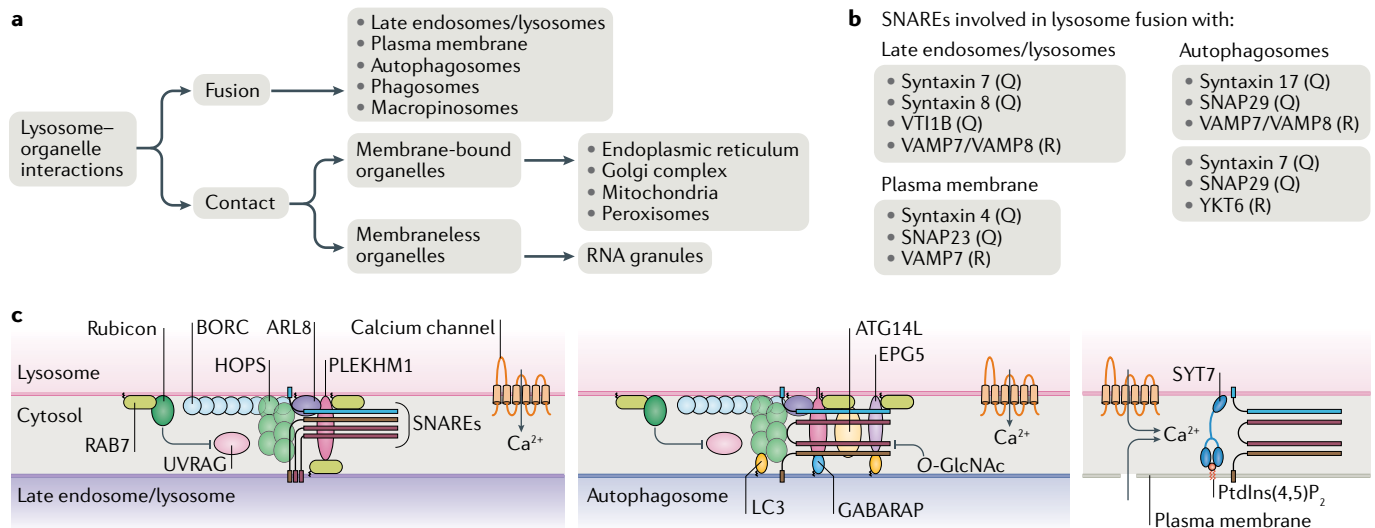


Fig. 4 | Lysosome-organelle interactions. **a** | Summary of interactions of lysosomes with other organelles (both membrane-bound and membraneless). **b** | SNARE proteins involved in fusion of lysosomes with other organelles. SNAREs assemble into *trans*-SNARE complexes comprising a four α -helix bundle at its core. On the basis of the presence of a Q or R residue within the 'zero' layer of the bundle, SNAREs are classified as Q-SNAREs (Q) or R-SNAREs (R). **c** | Machineries involved in fusion of lysosomes with different organelles. Assembly of distinct sets of SNAREs drives fusion of lysosomes with different organelles. Additional factors such as small GTPases (ARL8, RAB7), tethering factors (HOPS, EPG5, PLEKHM1, ATG14L), autophagosome-docking proteins (LC3, GABARAP), regulators (synaptotagmin 7 (SYT7)), BLOC1-related complex (BORC), UVRAG, Rubicon, O-linked N-acetylglucosamine (O-GlcNAc) modification and Ca²⁺ fluxes, contribute to the specificity, efficiency and regulation of particular fusion events. PtdIns(4,5)P₂, phosphatidylinositol 4,5-bisphosphate.

A more complex lysosome fusion event is the merger of lysosomes with autophagosomes in the process of autophagy¹¹⁴ (FIG. 4c). Whereas early studies of autophagy focused mainly on the mechanisms of induction and autophagosome formation, in recent years there has been growing interest in the role of the lysosome in autophagic degradation. This role is indeed critical, as lysosomes provide the hydrolases and the acidic pH necessary to degrade the autophagic substrates. Lysosomes also contribute a subset of SNAREs involved in fusion (FIG. 4b,c). Furthermore, lysosomes serve as platforms for various effectors and regulators of autophagosome-lysosome fusion, some of which are similar to those involved in lysosome-late endosome fusion (FIG. 4c); these effectors include the heterooctameric BLOC1-related complex (BORC) that promotes sequential recruitment of ARL8 and HOPS¹¹⁵, and RAB7, which recruits the tethering proteins PLEKHM1 (REF.¹¹¹) and EPG5 (REF.¹¹⁶), as well as the negative regulator of fusion events, Rubicon¹¹⁷ (FIG. 4c). The lysosome-tethered HOPS, PLEKHM1 and EPG5 in turn interact with phosphatidylethanolamine-conjugated (lipidated) forms of the Atg8/LC3/GABARAP family of docking proteins on the autophagosomal membrane, bringing together the lysosomal and autophagosomal membranes. Lastly, recent studies showed that autophagosome-lysosome fusion is negatively regulated by mTORC1, which catalyses phosphorylation of the tumour suppressor protein UVRAG, leading to its enhanced interaction with Rubicon and decreased interaction with HOPS, and thereby inhibiting fusion events¹¹⁸.

Lysosomes can also fuse with the plasma membrane by a process referred to as 'lysosomal exocytosis' (FIG. 4c). This process mediates a number of important lysosomal

functions, such as plasma membrane repair³³, formation of invasive protrusions in cancer cells¹¹⁹ and secretion of lysosomal contents into the extracellular space³⁶, as happens in the process of bone resorption¹²⁰.

Maintenance of cellular homeostasis requires that lysosome fusion events are followed by lysosome re-formation from hybrid organelles¹²¹. For example, in the process of autophagic lysosome re-formation, prolonged starvation induces the mTORC1-dependent extension of protolysosomal tubules from autolysosomes, which eventually detach and fuse to form new lysosomes¹¹. Likewise, re-formation of lysosomes after their fusion with phagosomes (phagolysosome resolution) also involves extension of protolysosomal tubules¹²² and regulation by mTORC1 (REF.¹²³). The molecular mechanisms involved in these re-formation processes, as well as in other lysosome tubulation and fission events, are still poorly understood¹²¹.

Lysosome contact sites. Over the past few years, it has been realized that lysosomes can also engage in non-fusogenic contacts with other membrane-bound organelles, such as the ER, peroxisomes, Golgi complex and mitochondria (FIG. 4a). These interactions involve the formation of membrane contact sites — organellar domains where closely apposed membranes are held together by various tethering proteins¹²⁴ (FIG. 5).

Lysosome-organelle contacts have important functional consequences. For example, maturation of early endosomes to late endosomes and then lysosomes was shown to be accompanied by increased formation of contacts with the ER¹²⁵. These contacts cause rearrangement of ER tubules¹²⁵, while also contributing to

Atg8/LC3/GABARAP family
Homologues of yeast autophagy-related protein 8 (Atg8); ubiquitin-like proteins that function in cargo recruitment to autophagosomes and autophagosome-lysosome fusion.

the budding of recycling endosomal tubules¹²⁶. Another major function of lysosome–ER membrane contact sites is the non-vesicular transfer of lipids between these organelles. For example, free cholesterol generated by hydrolysis of cholesterol esters in the lumen of lysosomes is exported out of the lysosome by the concerted action of the proteins NPC1 and NPC2 (FIG. 5).

Subsequent transfer of free cholesterol into the ER is thought to occur at membrane contact sites and to be mediated by lipid-transfer proteins such as ORP5 and ORP1L, and ER-resident proteins such as VAPA and VAPB¹²⁷ (FIG. 5). Another lipid-transfer protein, STARD3, mediates cholesterol transport in the opposite direction — from the ER to endolysosomes¹²⁸.

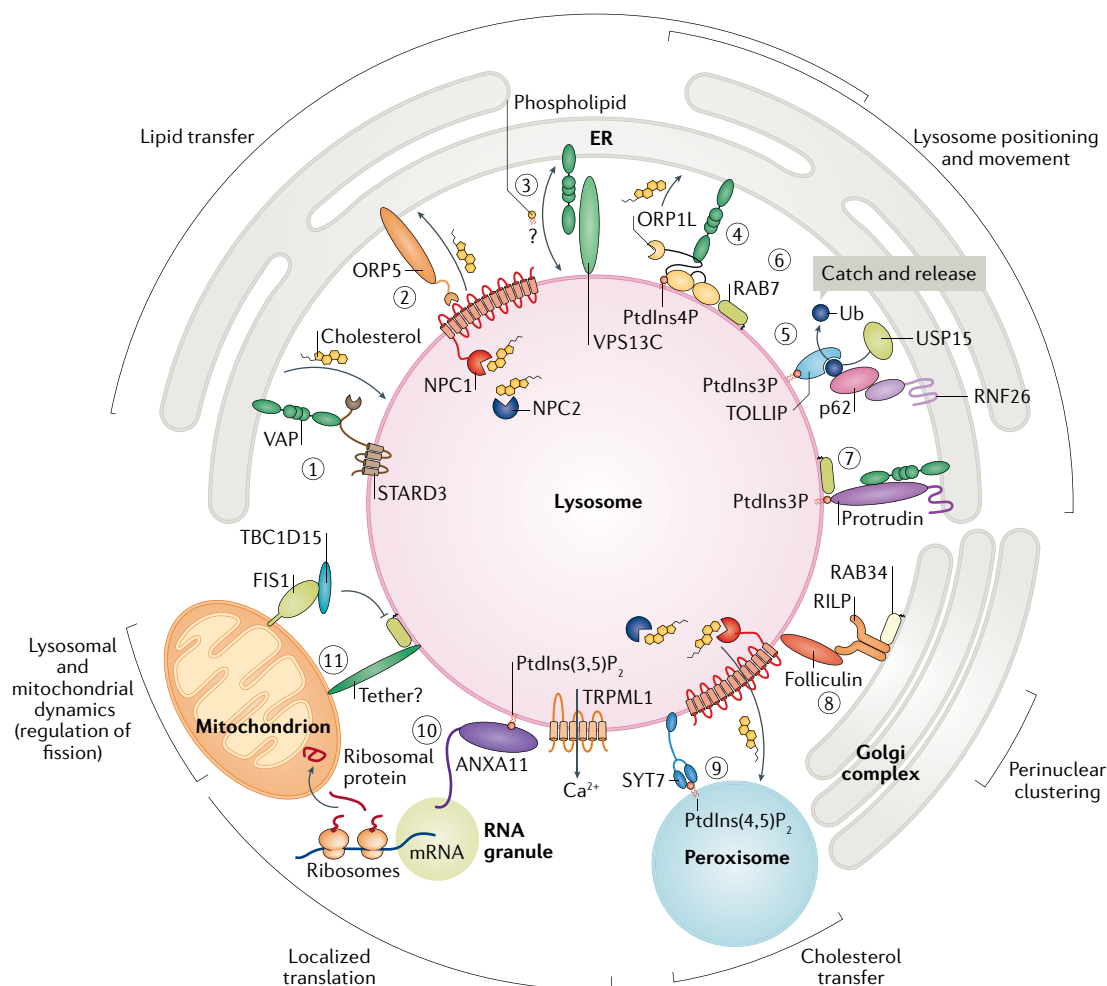


Fig. 5 | Membrane contacts between lysosomes and other organelles. Lysosomes establish versatile contacts with various organelles. Several of these contacts now have well-established functional consequences. An important function of contacts with the endoplasmic reticulum (ER) is lipid transfer, likely mediated by STARD3–VAP (step 1), ORP5–NPC1 (step 2), VPS13C–VAP (step 3) and/or RAB7–ORP1L–VAP (step 4) (of note, VAP exists as VAPA and VAPB paralogue with at least partially redundant functions)^{127–129}. STARD3, ORP5, Niemann–Pick type C1 protein (NPC1) and ORP1L serve as lipid transfer proteins. VAPs are ER-resident proteins and, other than for their contribution to membrane contacts, their roles in lipid transport processes are not clear. Lysosome–ER contacts also control lysosome positioning and movement. This can occur by the dynamic attachment of lysosomes to perinuclear ER via the RNF26–p62–TOLLIP–USP15 system. Here, lysosomes can be tethered to the ER via p62 in a manner that is regulated by the ubiquitylation status of p62, which is in turn dynamically modulated by the opposing activities of the ubiquitin (Ub) ligase RNF26 and the deubiquitylating enzyme USP15 (step 5)¹³¹. Proteins at lysosome–ER contacts also regulate coupling of lysosomes to cytoskeletal motors. For example, ORP1L regulates the cholesterol-dependent coupling of lysosomes to dynein–dynactin via RAB7 and RILP (step 6)¹³⁰, whereas protrudin regulates the coupling of lysosomes to kinesin 1 via RAB7 and FYCO1 (step 7)¹⁵⁴ (FIG. 6). Contacts with the Golgi complex via RAB34, RILP and folliculin promote perinuclear clustering of lysosomes in response to nutrient starvation (step 8)¹³⁴. Contacts with peroxisomes via synaptotagmin 7 (SYT7) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) mediate NPC1-dependent cholesterol transfer (step 9)¹³³. Annexin 11 (ANXA11)-mediated contacts promote the axonal transport of RNA granules for local translation of mRNAs encoding mitochondrial proteins (step 10)^{140,141}. Finally, lysosomes establish dynamic contacts with mitochondria, where tethering is promoted by RAB7 and counteracted by FIS1, which inhibits RAB7 via the activity of the RAB7 GTPase-activating protein TBC1D15 (step 11)¹³⁶. For a more detailed description of these mechanisms, see REFS^{3,114,124}. PtdIns3P, phosphatidylinositol 3-phosphate; PtdIns4P, phosphatidylinositol 4-phosphate; PtdIns(3,5)P₂, phosphatidylinositol 3,5-bisphosphate.

RHEB

Member of the RAS family of small GTPases that is mainly involved in activation of mechanistic target of rapamycin complex 1.

Retrograde movement

In general, movement from the plus end to the minus end of microtubules; in the axon, movement from the axon terminal towards the cell body.

Anterograde movement

In general, movement from the minus end to the plus end of microtubules; in the axon, movement from the cell body towards the axon terminal.

This reverse transport process is independent of the global transcriptional control of cholesterol-regulated genes and might serve to maintain cholesterol homeostasis in endolysosomal organelles without the need for more cholesterol synthesis¹²⁸. The protein VPS13C has recently been shown to interact with VAPs at lysosome–ER membrane contact sites¹²⁹ (FIG. 5). VPS13C has an amino-terminal lipid-binding domain that transfers phospholipids between lysosomes and the ER¹²⁹. The direction and function of this transfer, however, have not been established. In addition to lipid transfer, a number of proteins at lysosome–ER membrane contact sites have been implicated in the regulation of lysosome positioning and movement (see the section Lysosome positioning and motility), in some cases in response to changes in cholesterol or phosphatidylinositol 3-phosphate (PtdIns3P) levels^{130–132} (FIG. 5).

Lysosomes have also been shown to transfer free cholesterol to peroxisomes through membrane contact sites involving lysosomal synaptotagmin 7 and peroxisomal PtdIns(4,5)P₂ (REF.¹³³) (FIG. 5). An additional membrane contact site between the lysosome and the Golgi complex contributes to perinuclear clustering of lysosomes and consequent mTORC1 regulation by a non-lysosomal pool of its activator RHEB^{134,135}. This non-lysosomal pool of RHEB is at least in part associated with the Golgi complex itself, and is thought to contribute to mTORC1 activation at lysosome–Golgi contact sites¹³⁵. Finally, lysosomes form contact sites with mitochondria¹³⁶. These contacts are promoted by lysosome-associated RAB7 and counteracted by mitochondria-associated FIS1 through recruitment of the RAB7 GTPase-activating protein TBC1D15 (REF.¹³⁶). In addition to allowing control of lysosomal RAB7 activity by mitochondria, these contacts mark sites for mitochondrial fission, thereby allowing the regulation of both mitochondrial and lysosomal dynamics¹³⁶ (FIG. 5). Additional molecules have been shown to mediate contacts between the yeast vacuole (the equivalent of mammalian lysosomes) and other organelles^{137–139}, but the orthologous molecules have not yet been demonstrated to participate in lysosome contacts in mammalian cells.

Non-fusogenic lysosome interactions are not limited to membrane-bound organelles but have recently been found to include membraneless organelles such as RNA granules^{140,141} (FIG. 4). RNA granules are condensates of RNAs and proteins formed by liquid–liquid phase separation. Neuronal RNA granules were shown to be tethered by annexin 11 (ANXA11) to a subpopulation of lysosomes¹⁴¹. This tethering allows hitchhiking of the RNA granules on axonal lysosomes towards sites of local protein synthesis, particularly in association with axonal mitochondria and for synthesis of mitochondrial proteins^{140,141} (FIG. 5).

Lysosome positioning and motility

Another recent development in lysosome biology has been the realization that lysosomes are highly dynamic structures¹⁴². Lysosomes are spread throughout the cytoplasm, although with a higher concentration in the perinuclear region. At any given time, some lysosomes are stationary, while others move bidirectionally along microtubule

tracks. All lysosomes, however, are potentially motile, as over time some stationary lysosomes start to move, while some moving lysosomes stop moving. Several factors contribute to these dynamics, including immobilization by interactions with perinuclear ER¹³¹ and Golgi complex¹³⁴ (FIG. 5), or peripheral actin filaments underlying the plasma membrane¹⁴³, as well as mobilization by interaction with microtubule motors^{144,145} (FIG. 6). Whereas movement from the cell periphery towards the perinuclear region (retrograde movement) is mediated by coupling to cytoplasmic dynein and its activator dynactin^{145,146}, movement from the perinuclear region towards the cell periphery (anterograde movement) is mediated by coupling to several kinesins, including members of the kinesin 1 (KIF5) and kinesin 3 (KIF1) families^{144,147,148} (FIG. 6).

Regulation of lysosome positioning. Lysosome positioning and motility are subject to a variety of regulatory inputs. For instance, lysosomes redistribute towards the perinuclear region on removal of amino acids and/or serum (a source of growth factors) from the culture medium¹⁴⁹. The mechanisms that govern the perinuclear clustering of lysosomes during starvation are complex, involving effects on the interactions of lysosomes with other organelles and with microtubule motors. As an example of an effect on organelle contacts, serum and amino acid withdrawal induces the association of folliculin with lysosomes, thus initiating a chain of interactions with the dynein–dynactin adaptor RILP and the Golgi complex-localized GTPase RAB34 that ultimately tethers lysosomes to the perinuclear Golgi complex¹³⁴ (FIG. 5).

Nutrient deprivation also affects the interaction of lysosomes with microtubule motors, both enhancing dynein–dynactin-dependent retrograde transport and inhibiting kinesin-dependent anterograde transport. The enhancement of retrograde transport depends on at least two adaptor systems that independently couple lysosomes to dynein–dynactin: one involving TRPML1 and the Ca²⁺ sensor ALG2 (REF.¹⁵⁰), and the other comprising the lysosomal transmembrane protein TMEM55B and the adaptor protein JIP4 (REF.⁶⁵) (FIG. 6). Notably, nutrient starvation promotes TFEB/TFE3-regulated transcription of the genes encoding TRPML1 and TMEM55B (REFS^{61,65,66}), ensuring long-term enhancement of lysosome retrograde transport. However, because TFEB overexpression has an opposite effect of promoting peripheral redistribution of lysosomes³⁶, TFEB activity may also increase anterograde transport, the overall result being enhanced lysosome dynamics (that is, bidirectional transport between the centre and the periphery of the cell).

The inhibitory effects of starvation on anterograde transport involve other adaptor systems that couple lysosomes to kinesins. One such system consists of BORC and ARL8 (REFS^{148,151–153}) (FIG. 6). Regulator physically interacts with BORC, exerting a negative effect on the ability of BORC and ARL8 to recruit kinesins to lysosomes^{152,153}. Starvation strengthens the Regulator–BORC interaction¹⁵³, whereas epidermal growth factor stimulation — a potent cue for cell growth — weakens it¹⁵², resulting in lysosome redistribution towards the centre or the periphery of the cell, respectively (FIG. 6).

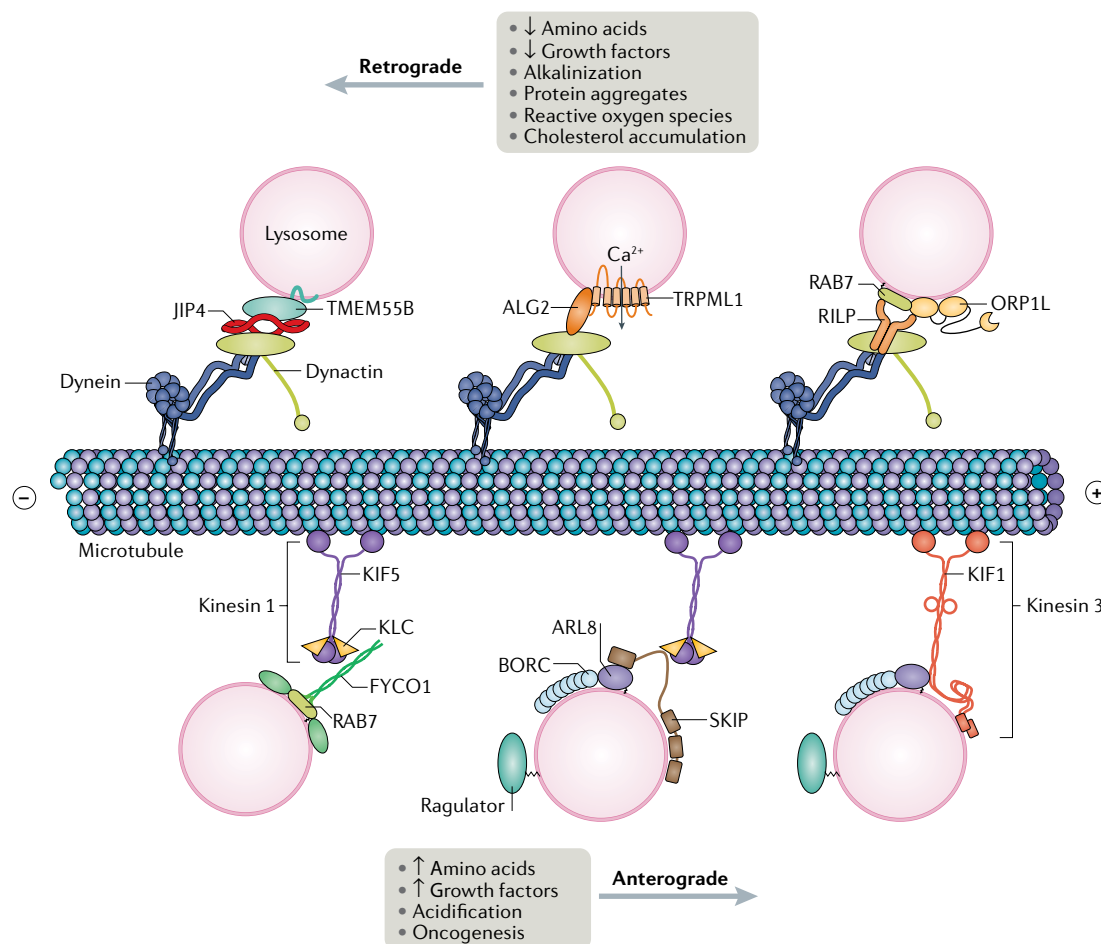


Fig. 6 | Machineries involved in lysosome motility. Several adaptor or scaffold complexes mediate coupling of lysosomes to dynein–dynactin or kinesins (predominantly kinesin 1 (composed of two KIF5 heavy chains and two light chains (KLC), and kinesin 3 (a homodimer of KIF1 heavy chains)) for retrograde and anterograde transport, respectively. These complexes are sensitive to environmental or metabolic conditions, allowing control of lysosome motility and positioning in response to different stimuli. The small GTPases RAB7 and ARL8 regulate lysosome motility and positioning in addition to lysosome interactions with other organelles (FIG. 4). RAB7 regulates coupling of lysosomes to both dynein–dynactin (via RILP) and kinesin 1 (via FYCO1)). ARL8 regulates coupling of lysosomes to kinesin 1 (via SKIP) and kinesin 3 (probably directly). For a more detailed description of these mechanisms, see REFS^{142,228}. BORC, BLOC1-related complex.

An additional system comprises the ER-anchored protein protrudin, which binds to RAB7 and PtdIns3P on the lysosomal membrane to promote the interaction of kinesin 1 with another PtdIns3P-binding adaptor, FYCO1, for movement of lysosomes towards the cell periphery¹⁵⁴ (FIG. 6). Amino acid starvation reduces PtdIns3P levels, dissociating protrudin and FYCO1 from lysosomes and thus causing perinuclear clustering of lysosomes¹³².

Functions influenced by lysosome positioning. In addition to allowing the response to changing nutrient levels, lysosome motility is critical for many other functions, including autophagy, microbial killing, antigen presentation, cell adhesion and migration, and cancer cell invasion¹⁵⁵. Several studies showed that lysosome positioning also influences mTORC1 signalling, albeit with variable results. In some studies, peripheral scattering of lysosomes induced by overexpression of ARL8, kinesins, FYCO1 or protrudin enhanced mTORC1 activity, whereas perinuclear clustering of lysosomes caused by

knockdown of the same proteins or BORC, or knock-out of the corresponding genes, reduced it^{132,149,156}. Since mTORC1 is activated by engagement of growth factor receptors at the plasma membrane, these effects were proposed to depend on the distance of lysosomes from the plasma membrane — the assumption being that the proximity of mTORC1 to the plasma membrane facilitates its activation. Other studies, however, showed that peripheral dispersal of lysosomes by inhibition of dynein¹⁵⁷ or acidification of the culture medium¹⁵⁸ inhibited mTORC1, whereas perinuclear clustering of lysosomes by human cytomegalovirus infection caused persistent mTORC1 activation¹⁵⁹. These effects were attributed to the spatial separation of lysosome-associated mTORC1 and the non-lysosomal pool of RHEB. The discrepancies among these findings may result from the different manipulations that were used to alter lysosome positioning, underscoring the complexity of the mechanisms that link lysosome positioning to mTORC1 signalling.

CORVET

Tethering complex composed of six subunits (VPS3, VPS8, VPS11, VPS16, VPS18 and VPS33) that functions in endosomal fusion events.

Neurofibrillary tangles

Intraneuronal aggregates of hyperphosphorylated tau protein that are most commonly associated with Alzheimer disease.

α -Synuclein

Presynaptic protein pathogenetically linked to Parkinson disease.

Pompe disease

Lysosomal storage disorder caused by acid α -glucosidase deficiency, which results in accumulation of glycogen in the cells.

Gaucher disease

Lysosomal storage disorder caused by deficiency of glucocerebrosidase, which results in the build-up of glucocerebroside in the cells.

Lewy bodies

Abnormal deposits of the protein α -synuclein in the brain of patients with various neurodegenerative disorders, including Lewy body dementia, Parkinson disease and Alzheimer disease.

Presenilin 1

One of the four subunits of the γ -secretase complex. The encoding gene is mutated in an inherited form of Alzheimer disease.

Lysosome dysfunction in disease

The rapidly expanding knowledge of lysosomal function has resulted in a better understanding of how lysosomal defects can lead to disease. While initially lysosomal dysfunction was identified in rare inherited conditions such as LSDs, more recent studies have uncovered a crucial role of defective lysosomal function in common disease entities such as neurodegenerative diseases, cancer and metabolic disorders. In particular, a decline in lysosomal function with age has been proposed to explain the prevalence of these diseases in elderly individuals^{160,161}.

Lysosomal storage disorders. The paradigmatic example of how dysfunctional lysosomes can cause human disease is represented by LSDs, a group of inherited monogenic diseases characterized by a progressive, multisystemic phenotype often associated with early-onset neurodegeneration. In these diseases, mutations in genes encoding either lysosomal resident proteins or non-lysosomal proteins involved in lysosomal function lead to an impairment of lysosome-mediated degradation and recycling processes, with progressive lysosomal accumulation of undegraded substrates^{162–166}.

LSDs may be caused by mutations in genes encoding lysosomal hydrolases, with consequent primary accumulation of a specific type of undegraded lysosomal substrate. LSDs belonging to this category share extensive similarities in terms of biochemical and cellular mechanisms^{162–166}. A more complex category of LSDs is represented by those caused by mutations in genes that encode lysosomal membrane proteins, which are characterized by broader cellular consequences². Examples of the latter category include mucopolipidosis type IV, which is caused by mutations in the *TRPML1* gene and is associated with major intracellular trafficking defects^{27,28} and Niemann–Pick disease type C1, which is caused by mutations of *NPC1* and is characterized by cholesterol accumulation in lysosomes¹⁶⁵.

LSDs may also be caused by mutations in genes that encode non-lysosomal proteins that are involved in the sorting, transport and post-translational modification of lysosomal enzymes. For example, mucopolipidosis type II is caused by mutations in the gene that encodes *N*-acetylglucosamine-1-phosphotransferase, which participates in the mannose 6-phosphate-mediated trafficking of lysosomal enzymes¹⁶². In multiple sulfatase deficiency, mutations in the sulfatase-modifying factor 1 gene (*SUMF1*), which encodes an ER-resident protein responsible for the post-translational modification of all sulfatases, result in a severe type of neurodegenerative LSD caused by the deficiency of several lysosomal as well as non-lysosomal sulfatases^{167,168}. Similarly, mutations in the gene that encodes the ER-associated protein CLN8, which is involved in the transport of lysosomal enzymes from the ER to the Golgi complex¹⁶⁹, cause a specific type of neuronal lipofuscinosis, an LSD associated with early-onset neurodegeneration and blindness¹⁶². Recent studies showed that LSDs may also be caused by mutated subunits of the endolysosomal multisubunit tethering complexes HOPS and CORVET such as VPS33A^{170–172}.

Initially, the pathogenesis of LSDs appeared simple and directly correlated to the primary storage of

accumulating lysosomal substrates. However, more recent studies based on genomic, cell biology and pathophysiology approaches have identified secondary pathways that have crucial roles in the disease phenotype. For instance, several studies have shown that in most LSDs there is a block of autophagy due to impaired fusion between autophagosomes and lysosomes^{173–175}. This block is caused by abnormal cholesterol accumulation in the lysosomal membrane and consequent reduction of the sorting and recycling of SNARE proteins¹⁷⁶. As a consequence of a block in autophagy, cells exhibit secondary accumulation of autophagy substrates such as aggregation-prone proteins and dysfunctional mitochondria, leading to inflammation and neurodegeneration, which are late-stage features of LSDs¹⁷⁴. Consistent with these observations, autopsy specimens from patients with LSDs showed neuropathological features that are typically detected in patients with common neurodegenerative diseases, such as phosphorylated tau aggregates, neurofibrillary tangles and accumulation of α -synuclein¹⁷⁴. These findings demonstrated a mechanistic link between the early-onset neurodegeneration found in LSDs and late-onset neurodegenerative diseases. In addition to neurodegeneration, defective autophagy also appears to be a major factor for LSD skeletal and growth abnormalities through impairment of collagen secretion and reduced bone growth¹⁷⁷.

Another important secondary consequence of lysosomal dysfunction in LSDs is the deregulation of mTORC1 activity. Recent studies using cellular and animal models showed that mTORC1 activity is perturbed in several LSDs. However, the nature of this perturbation appears to be cell type specific and disease specific. For example, in Pompe disease myofibers¹⁷⁸, as well as in a fly model of Gaucher disease¹⁷⁹, mTORC1 activity was reduced. Conversely, in chondrocytes from mouse models of mucopolysaccharidosis types VI and VII and of Niemann–Pick disease type C1, mTORC1 activity was increased¹⁷⁷. A possible explanation for these differences may be the nature of the primary and secondary substrates accumulating in different tissues in these LSDs. A notable example is represented by cholesterol, which is known to activate mTORC1 via an SLC38A9–NPC1 lysosomal signalling complex¹⁸. Consistently, inhibition of mTORC1 by either rapamycin or starvation significantly ameliorates lysosomal and autophagy defects in cellular models of both mucopolysaccharidosis type VII¹⁷⁷ and Niemann–Pick disease type C1 (REF.¹⁸⁰).

Neurodegenerative diseases. In addition to LSDs, lysosomal dysfunction has been identified in several common neurodegenerative diseases¹⁸¹, including late-onset forms of neurodegeneration (for example, Parkinson disease, Alzheimer disease and Huntington disease)¹⁸², amyotrophic lateral sclerosis¹⁸³, dementia with Lewy bodies¹⁸⁴ and the most common neuromuscular disorder, Charcot–Marie–Tooth disease^{185,186}.

Specific forms of common neurodegenerative diseases may be caused by mutations of genes involved in lysosomal function. Patients with Alzheimer disease who carry mutations in the presenilin 1 gene (*PSEN1*) show lysosomal and autophagic dysfunction¹⁸⁷ owing to

Amyloid precursor protein (APP). Transmembrane protein that is proteolytically processed to various products, including amyloid- β peptide involved in Alzheimer disease.

mTORC2

Protein complex comprising the mechanistic target of rapamycin kinase and five additional subunits (MLST8, DEPTOR, MSIN1, PROTOR and RICTOR) that functions in the regulation of cellular metabolism and growth.

Netrin

Secreted protein that functions in cell migration and cell–cell and cell–extracellular matrix interactions.

Anchor cells

Cells that participate in the development of the reproductive system in *Caenorhabditis elegans*.

defective lysosomal acidification and abnormalities of Ca^{2+} homeostasis^{188,189}. Likewise, a significant number of patients with Parkinson disease carry mutations in lysosomal–autophagic genes, which represent predisposing factors for disease pathogenesis^{190,191}. Importantly, heterozygosity for mutations in the gene encoding the lysosomal enzyme β -glucocerebrosidase (GBA), whose homozygous mutations cause the LSD Gaucher disease, is a major predisposing factor in Parkinson disease¹⁹².

Lysosomal–autophagic dysfunction may also contribute to the pathogenesis of common neurodegenerative diseases caused by protein aggregation. Indeed, in Parkinson disease and related ‘synucleinopathies’¹⁹³, impairment of the lysosomal–autophagic pathway contributes to α -synuclein accumulation and aggregation within Lewy bodies and neurites of neurons¹⁹⁴. In addition, it has been observed that defects in endolysosomal transport result in alterations of amyloid- β peptide production in Alzheimer disease¹⁹⁵.

Mounting evidence also suggests that protein aggregation itself may affect the lysosomal–autophagic pathway, thus generating a vicious cycle that boosts protein aggregation and toxicity. For example, in Huntington disease, the aggregation-prone protein huntingtin inhibits autophagosome biogenesis, transport and recognition of expanded huntingtin cargo^{196–198}, whereas in Parkinson disease α -synuclein aggregates can lead to lysosomal rupture and consequent cathepsin B-dependent increase in the level of reactive oxygen species¹⁹⁹. In addition, α -synuclein toxicity has been associated with a progressive decline of lysosomal function due to cytoplasmic retention of TFEB⁷¹. Furthermore, in Down syndrome (trisomy 21), a neurodevelopmental disorder that predisposes to early-onset Alzheimer disease, the extra copy of the gene that encodes amyloid precursor protein (APP) on chromosome 21 leads to increased production of the β -cleaved carboxy-terminal fragment of APP (APP- β -CTF or C99), which impairs lysosomal acidification and function through inhibition of the v-ATPase²⁰⁰. Finally, massive accumulation of lysosome-like organelles was detected at Alzheimer disease amyloid plaques, and in particular in regions where swollen axons contact amyloid deposits. This population of lysosomes had low luminal protease content, suggesting a block of endolysosomal maturation²⁰¹.

Together, these studies highlight the links between defects in lysosomal–autophagic pathways and the pathogenesis of neurodegeneration, thereby opening up potential therapeutic opportunities for targeting the lysosome to counteract these devastating diseases. In line with this, several studies have shown that in mouse models of neurodegenerative diseases, induction of lysosomal biogenesis and autophagy through viral-mediated overexpression of TFEB results in intracellular clearance of accumulating substrates and significant rescue of the disease phenotype²⁰².

Cancer. For a long time, the lysosome has been considered an attractive therapeutic target for cancer. This was mainly linked to evidence that induction of lysosome-dependent cell death^{39,203} is an effective therapeutic approach in a variety of cancer types²⁰⁴. However,

recent studies indicate that the role of the lysosome in cancer extends beyond its role in cell death and can be linked to its ability to fuel the increased demand of cancer cells for energy sources²⁰⁵. This is particularly important in conditions in which insufficient vascularization of tumours may limit nutrient availability. Several cancer types, such as pancreatic, lung, breast and prostate cancers, as well as glioblastoma and melanoma, have been shown to rely on the induction of lysosomal–autophagic degradation and recycling processes, which act as nutrient-scavenging pathways²⁰⁵.

Considering the role of the lysosomal–autophagic pathway in supporting proliferation and growth of cancer cells, it is not surprising that several types of cancer, including renal cell carcinoma, melanoma, alveolar soft part sarcoma and pancreatic adenocarcinoma, are associated with overexpression of mTOR-TFE genes^{206,207}. In these tumours, induction of mTORC1 by mTOR-TFE factors (see the earlier discussion in the section Regulation of TFEB by environmental cues) allows the concomitant hyperactivation of both autophagy-mediated nutrient-scavenging processes and the mTORC1-mediated biosynthetic pathway. Thus, the aberrant activation of both catabolic and biosynthetic pathways supports the energy-demanding metabolism of cancer cells⁹⁶.

Lysosomes also participate in other processes that promote cancer cell proliferation, invasion and metastasis. An acidic tumour microenvironment, in particular, has been shown to cause redistribution of lysosomes towards the cell periphery²⁰⁸, a process that may enhance proliferation through increased mTORC1 and mTORC2 signalling^{149,156}, as well as invasion and metastasis through exocytosis of lysosomal hydrolases²⁰⁹, matrix metalloproteinases²¹⁰ and integrins²¹¹. A recent study using *C. elegans* as a model system uncovered a novel mechanism by which lysosome exocytosis promotes cell invasion during normal development¹¹⁹. In this study, interaction of netrin with its receptor on anchor cells was shown to induce lysosome exocytosis leading to the formation of metalloproteinase-enriched protrusions that breach the basement membrane and thus allow the invasion of vulval tissue by the anchor cells¹¹⁹. The orthologue of netrin in humans, netrin 1, is overexpressed in various metastatic cancers, and also stimulates cancer cell invasion²¹², suggesting that a similar mechanism of lysosomal exocytosis may underlie the spreading of cancer cells. This role has prompted studies to identify pharmacologic agents that interfere with lysosome dispersal towards the cell periphery as a potential cancer therapy²¹³.

Metabolic disorders. Lysosomes have also been implicated in the pathogenesis of various metabolic disorders, as best exemplified by their roles in obesity and diabetes^{214–216}. The lipid overload that is characteristic of obesity impairs lysosomal function by various mechanisms²¹⁴. For instance, feeding of mice with a high-fat diet inhibits lysosomal acidification and acid hydrolase activity, and triggers permeabilization of the lysosomal membrane, with consequent impairment of lysosomal and autophagic functions in different tissues²¹⁷. Despite the harmful effects of a high-fat diet, lysosomal adaptation

via TFEB and TFE3 can exert a protective effect on whole-body lipid and glucose homeostasis, thereby lessening the tendency towards obesity and diabetes⁶⁷.

Lysosomes also participate in whole-body glucose regulation by maintaining the fitness of insulin-producing pancreatic β -cells²¹⁵. An early response of these cells to nutrient starvation is the degradation of nascent insulin granules by sequestration into Golgi complex-derived double-membraned structures that subsequently fuse with lysosomes — a process distinct from canonical autophagy²¹⁸. Lysosomal degradation of nascent insulin granules decreases the secretion of insulin and provides the β -cells with an alternative source of amino acids during fasting. When subjected to a high-fat diet, β -cells induce canonical autophagy, which protects them from ER stress and other harmful effects of lipid oversupply²¹⁹. However, prolonged lipid overload overwhelms the protective effects of lysosomes and autophagy on metabolic homeostasis and eventually leads to lysosome damage, resulting in deterioration of lysosomal function and β -cell loss.

Conclusions and perspective

The studies reviewed here make it clear that the degradative function of lysosomes is intimately linked to multiple pathways that control cell and organismal homeostasis. Past are the days when lysosomal function could be studied in isolation from the functions of other cellular organelles and processes. Instead, current studies must address the functions of lysosomes in the context of whole cells and organisms, in all of their broad diversity.

Despite the explosion in the understanding of the connection between lysosomes and other cellular structures and pathways, current knowledge of this connection may just represent the tip of the iceberg. Indeed, the lysosomal membrane comprises hundreds of integral and peripheral proteins, many of which are of unknown function. What other nutrient and ion transporters, scaffolds and signalling proteins might connect lysosomal function to cellular metabolism? What other lysosome signalling pathways exist in specialized cell types? In addition to the pathways that were reviewed here (transcriptional regulation by MiT-TFE, BRD4, STAT3 and ZKSCAN3 factors), are there other pathways that mediate global adaptation of lysosomal function to environmental, developmental or pathological cues? Do lysosome-related organelles such as cytotoxic T cell granules, melanosomes and platelet dense bodies share signalling pathways with lysosomes or do they

have their own, distinct pathways? Even in cells that do not have specialized lysosome-related organelles, there is evidence for heterogeneity of lysosomes. For example, lysosomes can exhibit different size, shape, positioning, motility, luminal pH, acid hydrolase content and other properties^{131,150,155,220–224}. Do these various lysosome subtypes have different functions? Are there specific populations of lysosomes involved in different cellular functions, for example signalling versus exocytosis? Finally, the ‘life cycle’ of lysosomes remains poorly understood. For example, the understanding of how they fuse and interact with other organelles is still incomplete. Even less well understood is how lysosomes fission and how they re-form after fusing with autophagosomes, phagosomes or other hybrid organelles¹²¹. These questions are just examples of all the exciting work that lies ahead to fully elucidate the multiple roles of lysosomes within the cell.

Also promising are the prospects for applying the knowledge gained from fundamental studies of lysosome biology to the treatment of lysosome-related diseases. Considerable advances have already been made in the therapy for LSDs by replacement of the defective lysosomal hydrolases (enzyme replacement) and use of small molecules that inhibit the synthesis of the accumulating substrates (substrate reduction) or promote the folding and stability of mutant lysosomal hydrolases (with the use of chemical chaperones)^{162–165}. Some LSDs have also been successfully treated by gene therapy to express the normal form of the defective lysosomal hydrolase^{162–165}. A current challenge is to translate knowledge of lysosome biology to the treatment of more common disorders such as neurodegeneration, cancer and metabolic diseases. In this regard, the use of mTOR inhibitors is being explored to enhance autophagy in neurodegenerative diseases caused by accumulation of protein aggregates²²⁵. Lysosomotropic agents such as chloroquine and hydroxychloroquine are being repurposed to inhibit lysosomal degradation and induce lysosome-dependent cell death in cancer cells²²⁶. Autophagy inhibitors and lysosomotropic agents may also enhance insulin secretion by pancreatic β -cells in certain diabetic conditions²²⁷. As knowledge of the multiple functions of lysosomes in the maintenance of cellular homeostasis expands, we can look forward to the development of better therapeutic interventions for these and other lysosome-related disorders.

Published online 25 November 2019

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Acknowledgements

The authors thank A. De Matteis, G. Diez-Roux, P. Luzio, R. Mattera, G. Napolitano and C. Settembre for comments on the manuscript and J. Goodwin for the original draft of Fig. 3a. Work in A.B.'s laboratory is supported by the US National Institutes of Health (R01-NS078072), the Italian Association for Cancer Research (AIRC) (IG 2018 22103), Fondation Louis-Jeantet and the Telethon Foundation. Work in J.S.B.'s laboratory is supported by the Intramural Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health (ZIA HD001607).

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

A.B. is a co-founder of CASMA Therapeutics (Boston, MA, USA). J.S.B. declares no competing interest.

Peer review information

Nature Reviews Molecular Cell Biology thanks R. Puertollano, S. Ferguson, J. Klumperman and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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