Signals from the lysosome: a control centre for cellular clearance and energy metabolism

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Abstract | For a long time, lysosomes were considered merely to be cellular 'incinerators' involved in the degradation and recycling of cellular waste. However, now there is compelling evidence indicating that lysosomes have a much broader function and that they are involved in fundamental processes such as secretion, plasma membrane repair, signalling and energy metabolism. Furthermore, the essential role of lysosomes in autophagic pathways puts these organelles at the crossroads of several cellular processes, with significant implications for health and disease. The identification of a master regulator, transcription factor EB (TFEB), that regulates lysosomal biogenesis and autophagy has revealed how the lysosome adapts to environmental cues, such as starvation, and targeting TFEB may provide a novel therapeutic strategy for modulating lysosomal function in human disease.

Glycocalyx
The polysaccharide-based coating on the inner side of a lysosomal membrane that protects this organelle from digestion by lysosomal enzymes.

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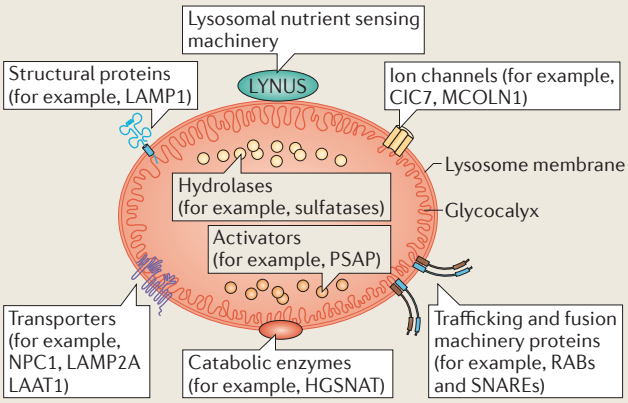
Lysosomes are membrane-bound organelles that were first described in 1955 by Christian de Duve[1]. They have an acidic lumen, which is limited by a single-lipid bilayer membrane and contains several types of hydrolases that are devoted to the degradation of specific substrates. The lysosomal membrane contains proteins that are involved in the transport of substances into and out of the lumen, acidification of the lysosomal lumen and fusion of the lysosome with other cellular structures[2]. Extracellular material that is destined for degradation reaches the lysosome mainly through the endocytic pathway[3], whereas intracellular components are transported to the lysosome by autophagy[4–6]. Lysosomes can also secrete their contents by fusing with the plasma membrane[7,8]. This process, which is known as lysosomal 'exocytosis', is very active in particular cell types, such as cells from the haematopoietic lineage[9], osteoclasts[10] and melanocytes[11]. In addition to cellular clearance and secretion, the lysosome mediates a range of biological processes, such as plasma membrane repair, cell homeostasis, energy metabolism and the immune response. Little is known about how lysosomal function varies in different cells, tissues, life stages and individuals, as well as under different physiological conditions. However, in recent years, the static view of the lysosome has progressively changed into a much broader and dynamic perspective. The ability of the lysosome to adapt to different environmental cues became evident with the discovery that lysosomal biogenesis and function are subject to global transcriptional regulation. This novel concept of 'lysosomal adaptation' is important for our understanding of how basic biological processes, ranging from cellular clearance to the control of energy metabolism, respond to environmental cues.

In this Review, we first describe the structure of the lysosome and its established role in cellular clearance. We then consider the emerging roles of lysosomes, including their function in nutrient sensing and signalling, before discussing the identification of the transcription factor EB (TFEB) as a key protein that regulates lysosomal biogenesis and autophagy[12,13]. Finally, we focus on how lysosomal dysfunction leads to human disease.

Lysosome structure

The complex series of events leading to the formation of a mature lysosome has been described in recent articles[2,14–21]. The mature lysosome has an acidic lumen encircled by a cholesterol-poor membrane[22] (BOX 1). The main function of the lysosomal membrane is to segregate the 'aggressive' acidic environment of the lumen from the rest of the cell. This is ensured by the presence of a thick glycocalyx that lines the internal perimeter to prevent the lysosomal membrane being degraded by luminal acid hydrolases. The lysosomal membrane also actively mediates the fusion of lysosomes

Box 1 | **The structure of the lysosome**



Lysosomes have a limiting membrane, which is composed of a single-lipid bilayer and integral and peripheral proteins, and an acidic lumen that contains soluble hydrolytic enzymes and activators[47–49,194,195]. A glycocalyx lines the internal lysosomal perimeter, protecting the membrane from the acidic environment of the lumen. Soluble enzymes are directly involved in the degradation of metabolites, whereas the lysosomal membrane segregates this catalytic potential and also actively participates in the maintenance of plasma membrane integrity, the establishment of the acidic pH of the lysosomal lumen, metabolite and ion transport, lysosomal trafficking and catalysis. Some key functional categories of lysosomal membrane proteins are shown (see the figure). Trafficking and fusion machinery proteins comprise SNAREs and RABs. Structural proteins include lysosome-associated membrane protein 1 (LAMP1), which is the most abundant lysosomal membrane protein accounting for 50% of total protein in this membrane. LAMP1 is mainly involved in lysosomal trafficking by mediating the attachment of lysosomes to the transport machinery[2,196]. The lysosomal nutrient sensing (LYNUS) machinery includes several protein complexes that interact on the lysosomal surface, and its role is to sense the nutrient content of the lysosome and signal the information to the nucleus (see main text). An important component of the LYNUS machinery is the vacuolar ATPase (v-ATPase), a large multimeric channel that uses the energy derived from ATP hydrolysis to transport protons across the lysosomal membrane in order to generate the acidic pH of the lysosomal lumen[197,198]. Several ion channels have been identified on the lysosomal membrane. The transient receptor potential (TRP) family member mucolipin 1 (MCOLN1) is a non-selective cation channel[199] that is involved in Ca^{2+} signalling during lysosomal fusion with other membranes, such as the plasma membrane[85–87] and autophagosomes[200]. A deficiency in MCOLN1 causes mucolipidosis type IV, a lysosomal storage disease[201,202]. CIC7, a Cl^- channel, contributes to lysosomal acidification and is involved in inherited osteopetrosis[60,61,203]. Transporters in the lysosomal membrane include LAMP2A, which mediates chaperone-mediated autophagy by binding cytosolic protein substrates on the lysosomal membrane so that they can be internalized and degraded[5,204]. Mutations of LAMP2A cause Danon disease, which is associated with the accumulation of autophagic vacuoles in muscle cells[205]. Niemann–Pick C1 protein 1 (NPC1) is a lysosomal membrane protein involved in the export of cholesterol from the endolysosomal compartment, and it is mutated in Niemann–Pick disease type C1 (REF. 206). The recently identified lysosomal amino acid transporter 1 (LAAT1) is involved in the transport of Lys and Arg across the lysosomal membrane and into the lysosome, and it has a crucial role in cellular amino acid homeostasis[207]. Enzymes on the lysosomal membrane include heparan-α glucosaminide *N*-acetyltransferase (HGSNAT). This enzyme participates in the stepwise degradation of heparan sulphate[208–210], and mutation of this protein causes mucopolysaccharidosis type IIIC. PSAP, prostate-specific acid phosphatase.

with other cellular structures, such as late endosomes, autophagosomes and the plasma membrane, as well as the transport of metabolites, ions and soluble substrates into and out of lysosomes.

Lysosomal trafficking and fusion are mediated by specific sets of membrane-associated RAB GTPases[17,18,23,24] and SNARE proteins[25–27]. Of note, the ability of minimal 'synthetic' endosomes to fuse *in vitro* with purified early endosomes or with each other was reconstituted by using 17 recombinant human proteins, including specific RAB GTPases and SNAREs[28]. RAB5 and RAB7 are specifically involved in the tethering and docking processes in endolysosomal membrane trafficking pathways[23,24]. Furthermore, a reduction in RAB5 levels decreases the number of endosomes and lysosomes and blocks endocytosis[29]. A specific combinatorial set of SNAREs, including vesicle-associated membrane protein 7 (VAMP7), VAMP8, VTI1B, syntaxin 7 and syntaxin 8, forms the *trans*-complexes that drive lysosome–endosome fusion and the homotypic fusion between endosomes[25]. Interestingly, recent studies revealed that SNAREs that are involved in the fusion between autophagosomes and endolysosomal vesicles, such as syntaxin 17, also participate in autophagosome biogenesis[30,31].

The lysosomal lumen contains approximately 60 different soluble hydrolases, which are active at acidic pH. These enzymes are the main players in the execution of multistep catabolic processes. They include members of protein families such as the sulphatases, glycosidases, peptidases, phosphatases, lipases and nucleases, which allow the lysosome to hydrolyse a vast repertoire of biological substrates, including glycosaminoglycans, sphingolipids, glycogen and proteins. The targeting of most lysosomal enzymes to lysosomes, as well as their ability to be secreted and taken up again by cells, is mediated by a mannose-6-phosphate modification that they undergo in the late Golgi compartments[14,32]. The ability of cells to take up lysosomal enzymes via the mannose-6-phosphate receptor (MPR) is the basis for enzyme replacement therapy for several lysosomal storage diseases (LSDs)[33]. A different targeting mechanism, which is mediated in part by the lysosomal receptor LIMP2 (lysosome integral membrane protein 2; also known as SCARB2), was recently identified for β-glucocerebrosidase[34].

Autophagosomes
Intracytoplasmic vacuoles that contain elements of the cytoplasm of a cell. They fuse with lysosomes, and the contents are subjected to enzymatic digestion.

Danon disease
An X-linked dominant disorder caused by mutations in the gene encoding lysosome-associated membrane protein 2 (LAMP2). It predominantly affects cardiac muscle.

Niemann–Pick disease type C1
An autosomal recessive lipid storage disorder that is caused by mutation in the *NPC1* (Niemann Pick type C1) gene. It is characterized by progressive neurodegeneration.

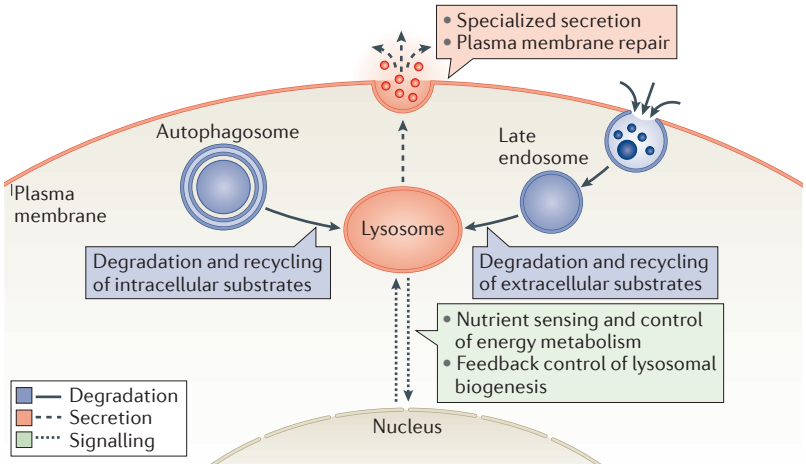


Figure 1 | **Main functions of the lysosome and their relationship with key cellular processes.** Lysosomes are involved in the degradation and recycling of intracellular material (via autophagy) and extracellular material (via endocytosis). In these processes, lysosomes fuse with autophagosomes and with late endosomes, respectively. The resulting breakdown products are used to generate new cellular components and energy in response to the nutritional needs of the cell. Lysosomes also undergo Ca^{2+} regulated exocytosis to secrete their content into the extracellular space and to repair damaged plasma membranes. Upon plasma membrane injury, lysosomes rapidly migrate to the damaged site and fuse with the plasma membrane to allow efficient resealing. More recently, lysosomes have been identified as signalling organelles that can sense nutrient availability and activate a lysosome-to-nucleus signalling pathway that mediates the starvation response and regulates energy metabolism.

The selective degradation of intra-lumenal membranes and lipids within lysosomes occurs in specialized intra-lysosomal vesicles. These vesicles contain a complex machinery composed of proteins that are involved in lipid degradation, such as water-soluble acid hydrolases and sphingolipid activator proteins (SAPs)[35–39]. Studying glycosphingolipid catabolism in patients with defects in this process was instrumental for the understanding of this complex pathway[40–42].

Importantly, a number of non-lysosomal proteins modulate the functions of lysosome-resident proteins. Prominent examples of these are the two different types of MPRs, cation-independent MPR (CI-MPR) and cation-dependent MPR (CD-MPR), which dynamically shuttle between the *trans*-Golgi network (TGN) and late endosomes and are involved in the targeting of lysosomal enzymes to the lysosome[32]. Another example is sulphatase-modifying factor 1 (SUMF1), an endoplasmic reticulum (ER)-resident protein that is responsible for a post-translational modification (PTM), the conversion of a highly conserved Cys in the active site to α-formylglycine, which is required for the activation of all sulphatases[43,44].

Various methods have been used to purify lysosomes and analyse their proteome[45–49]. Some of these approaches are based on subcellular fractionations, whereas others are based on specific features of soluble lysosomal proteins, such as the mannose-6-phosphate modification of their carbohydrate moieties[49]. In these efforts, it has been difficult to distinguish between lysosome-resident proteins, which are constituents of the lysosomal machinery, and proteins that are delivered to the lysosome for degradation. Therefore, we are still far from the identification and functional characterization of all lysosomal proteins. On the basis of current data, a little over 100 bona fide lysosome-resident proteins have been identified; ~70 of these are lysosomal matrix proteins and ~50 are lysosomal membrane proteins[48]. However, these numbers are likely to increase in the near future.

Lysosome functions

Lysosomal functions can be schematically divided into three main types: degradation, secretion and signalling (FIG. 1).

Lysosome-mediated degradation. Similar to the transport of urban waste to incinerators, the collection and transport of cellular waste to lysosomes requires complex logistics. The cell has developed different routes for transporting extracellular and intracellular waste to the lysosome. Extracellular material reaches the lysosome mainly through endocytosis. The capture of extracellular material and integral membrane proteins occurs through specific endocytic mechanisms according to the nature of the cargo. Prominent examples of endocytosis are phagocytosis, macropinocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis and clathrin- and caveolin-independent endocytosis[50]. Signalling receptors may undergo endocytosis through clathrin-mediated endocytosis[51] or clathrin-independent mechanisms[52]. After internalization, the receptors are routed to early endosomes[53]. From the endosomes, the receptors can either be recycled back to the plasma membrane to allow for repeated receptor activation or be sorted and targeted for lysosomal degradation, resulting in the termination of receptor signalling[54–56].

A known hallmark of endosome-to-lysosome maturation is the progressive decrease of the internal pH to ~pH 5 in the mature lysosome[57]. This is crucial for the uptake of acid hydrolases by MPRs into the endosomal lumen and the recycling of receptors back to the Golgi network[15]. The generation and maintenance of the lysosomal pH gradient requires the activity of a proton-pumping v-type ATPase, which uses the energy of ATP hydrolysis to pump protons into the lysosomal lumen[58]. Additional lysosomal membrane channels are thought to be involved in lysosomal acidification, such as the anion transporter chloride channel 7 (ClC7)[59–62] and the cation transporters mucolipin 1 (MCOLN1; also known as TRPML1) and two pore calcium channel 1 (TPC1) and TCP2 (REF. 62), which mediate Ca^{2+} and Na^{+} release from the lysosome[62–65, 106]. However, the role of each of these channels and the precise mechanisms underlying the complex regulation of lysosomal acidification and ion balance are still controversial and require further investigation.

Intracellular materials reach the lysosome through the process of autophagy, a 'self-eating' catabolic pathway that is used by cells to capture their own cytoplasmic components destined for degradation and recycling. Three types of autophagy have been identified: microautophagy; chaperone-mediated autophagy (CMA); and macroautophagy. During microautophagy, cytosolic

proteins are engulfed in the lysosome through the direct invagination of lysosomal or endosomal membranes[6,66,67]. In CMA, cytosolic proteins are transported into the lysosomal lumen through chaperone- and receptor-mediated internalization, which requires the unfolding of proteins and their translocation via lysosome-associated membrane protein 2A (LAMP2A)[5,68,69]. Macroautophagy, herein referred to as autophagy, relies on the biogenesis of autophagosomes, which are double membrane-bound vesicles that sequester cytoplasmic material and then fuse with lysosomes. Thus, the role of all three types of autophagy in degradation and recycling processes is strictly dependent on lysosomal function.

Autophagy is activated by a broad range of cellular stress-inducing conditions and it mediates the degradation of protein aggregates, oxidized lipids, damaged organelles and intracellular pathogens. The resulting breakdown products are used to generate new cellular components and energy in response to the nutritional needs of the cell. The mechanisms underlying autophagy and its relevance both in health and disease have been extensively studied in the past decade and comprehensively described in recent reviews[70,71].

Lysosomal exocytosis. Lysosomes can secrete their content through a process called lysosomal exocytosis, which can be detected by the translocation of lysosomal membrane marker proteins (for example, LAMP1) to the plasma membrane[7,8,72]. In this process, lysosomes fuse with the plasma membrane through a Ca^{2+}-regulated mechanism that leads to a bulk release of the lysosomal content into the extracellular matrix[72–77]. Initially, lysosomal exocytosis was thought to be limited to 'professional' secretory cells that contain a subset of specialized lysosome-related organelles (LROs)[75], but soon it was shown that any cell type can perform this function[72]. Lysosomal exocytosis mediates several physiological processes, such as degranulation in cytotoxic T lymphocytes[78], bone resorption by osteoclasts[10], parasite defence by mast cells and eosinophils[79,80], melanocyte function in pigmentation[11], platelet function in coagulation[81] and hydrolase release by spermatozoa during fertilization[82].

The molecular machinery mediating Ca^{2+}-regulated exocytosis of conventional lysosomes includes the vesicle SNARE (v-SNARE) VAMP7, the Ca^{2+} sensor synaptotagmin VII (SYTVII) on lysosomes, the target SNAREs (t-SNAREs) SNAP23 and syntaxin 4 on the plasma membrane[83] and several RAB proteins on the lysosomal surface[8,27,83,84]. Another important mediator of lysosomal exocytosis is the lysosomal membrane Ca^{2+} channel MCOLN1 (REFS 85–87). It was also postulated that autophagy proteins may regulate lysosomal exocytosis. For example, lipidation of the late autophagosome marker LC3 is required for the secretion of lysosomal contents into the extracellular space, as this directs the lysosomes to fuse with the plasma membrane[88–90]. However, autophagosomes may not be mediating this process[89].

Lysosomal exocytosis is not only responsible for the secretion of lysosomal content, it also has a crucial role in plasma membrane repair. Plasma membrane injuries induce the rapid migration of lysosomes to the damaged site. Lysosomes then fuse to the plasma membrane and efficiently reseal the damaged sites[91,92]. This process is also important in defence mechanisms against bacterial infection[93] and has been implicated in a specific type of muscular dystrophy, which is characterized by a defect in muscle fibre repair[94].

Lysosomal exocytosis is transcriptionally regulated by TFEB, a master regulator of lysosomal biogenesis (see below). TFEB induces both the docking and fusion of lysosomes with the plasma membrane by regulating the expression of certain genes, the protein products of which increase lysosomal dynamics and cause a MCOLN1-mediated increase in intracellular Ca^{2+} (REF. 86). Interestingly, TFEB-mediated regulation of lysosomal exocytosis has an important role in osteoclast differentiation and bone resorption[95].

Signalling from lysosomes. It has become evident that the lysosome plays an important part in nutrient sensing and in signalling pathways that are involved in cell metabolism and growth. Remarkably, the kinase complex mammalian target of rapamycin complex 1 (mTORC1), a master controller of cell and organism growth[96], exerts its activity on the lysosomal surface[97]. The lysosomal localization of mTORC1 suggests a mechanistic co-regulation between cell growth and cell catabolism. Growth factors, hormones, amino acids, glucose, oxygen and stress are the major activators of mTORC1, which in turn positively regulates proteins, mRNAs, lipid biosynthesis and ATP production[96,98]. In this way, mTORC1 regulates the balance between biosynthetic and catabolic states. When nutrients are present, mTORC1 directly phosphorylates and suppresses the activity of the kinase complex ULK1–ATG13–FIP200 (unc-51-like kinase 1–autophagy-related 13–focal adhesion kinase family-interacting protein of 200 kDa[99–101]), which is required to induce autophagosome biogenesis[102,103]. The inhibition of mTORC1, by either starvation or drugs, leads to the activation of ULK1–ATG13–FIP200 and autophagy. Thus, the level of cellular autophagy inversely correlates with mTORC1 activity, and the pharmacological inhibition of mTORC1 potently stimulates autophagy.

It was recently shown that the level of amino acids inside the lysosomal lumen controls mTORC1 docking on the lysosomal surface, which is a prerequisite for its activity, and that amino acids must accumulate in the lysosomal lumen in order for mTORC1 to bind and become activated[104]. These observations support the idea that mTORC1 activity is dependent on the lysosome and explains why mTORC1 is reactivated upon the lysosomal degradation of autophagic substrates that occurs during starvation[105]. A recent study demonstrated that an endolysosomal ATP-sensitive Na^+-permeable channel, $lysoNa_{ATP}$, which is located on the lysosomal membrane, also interacts with mTORC1 and participates in nutrient sensing. During starvation, mTORC1 is released from the lysosomal surface, and $lysoNa_{ATP}$ is constitutively open. Thus, $lysoNa_{ATP}$ regulates lysosomal pH stability and amino acid homeostasis by responding to ATP levels and controlling the lysosomal membrane potential[106].

Lysosome-related organelles
(LROs). Cell type-specific compartments that include melanosomes, lytic granules, major histocompatibility complex class II compartments, platelet-dense granules, basophil granules, azurophil granules and *Drosophila melanogaster* pigment granules.

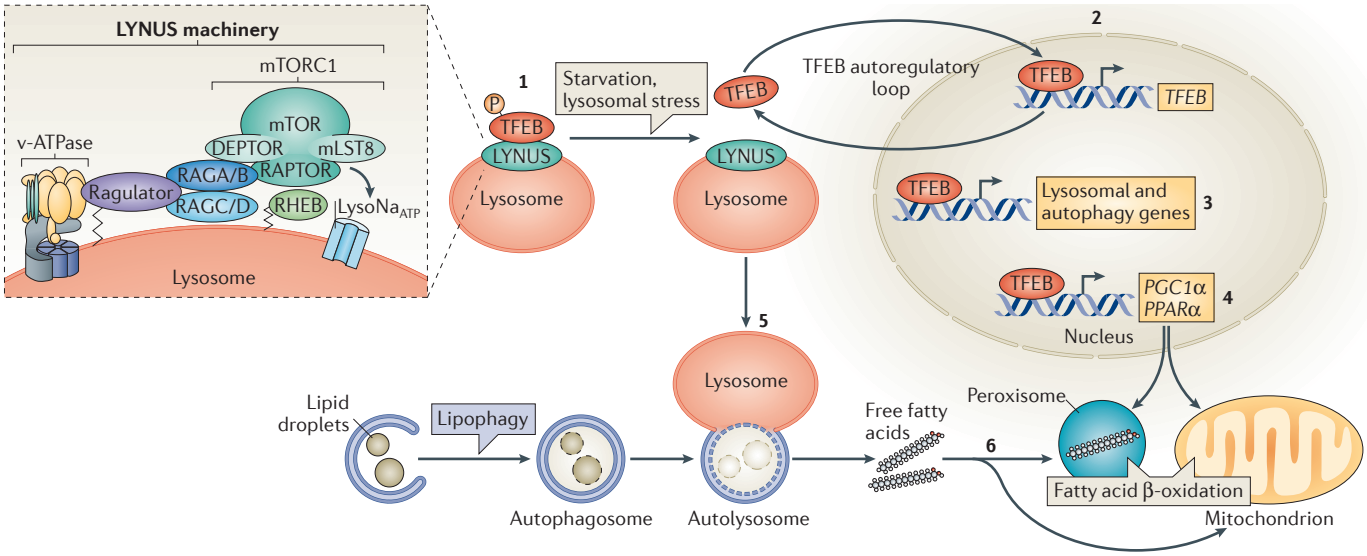


Figure 2 | **Model of TFEB regulation and function during starvation.** This model illustrates how the activity of transcription factor EB (TFEB) is induced by limited nutrient availability and mediates the starvation response by regulating lipid catabolism. In the presence of sufficient nutrients, TFEB interacts with the lysosome nutrient sensing (LYNUS) machinery, which senses lysosomal nutrient levels via the vacuolar ATPase (v-ATPase) complex, and is phosphorylated by mammalian target of rapamycin complex 1 (mTORC1) on the lysosomal surface (1). This keeps TFEB inactive by cytosolic sequestration. During starvation, mTORC1 is released from the LYNUS machinery and becomes inactive. Thus, TFEB can no longer be phosphorylated by mTORC1 and translocates to the nucleus, where it induces its own transcription (2). Therefore, starvation regulates TFEB activity through a dual mechanism that involves a post-translational modification (that is, phosphorylation) and a transcriptional autoregulatory loop. Once in the nucleus, TFEB regulates the expression of genes involved in the lysosomal–autophagy pathway (3), as well as of PPARα (peroxisome proliferator-activated receptor-α) and PGC1α (PPARγ co-activator 1α) and their target genes (4). In this way, TFEB controls the starvation response by activating both lipophagy (5) and fatty acid β-oxidation (6). The insert shows the main components of the LYNUS machinery. mTORC1, which includes regulatory proteins associated with mTOR, such as RAPTOR (regulatory-associated protein of mTOR), mLST8 (mammalian lethal with SEC13 protein) and DEPTOR (DEP domain-containing mTOR-interacting protein) [211], physically interacts with RAG GTPases (RAGA or RAGB and RAGC or RAGD), which activate mTORC1 on the lysosomal surface[212]. A complex known as Ragulator mediates the activation and docking of RAG GTPases to the lysosomal membrane[97,213]. The small GTPase RAS homologue enriched in brain (RHEB) is also involved in the growth factor-mediated activation of mTORC1 (REFS 214,215). The v-ATPase complex functions in amino acid sensing and mediates amino acid-sensitive interactions between RAG GTPases and Ragulator, which is the initial step in lysosomal signalling[104]. The endolysosomal ATP-sensitive Na^+-permeable channel (lysoNa$_{ATP}$), which comprises the subunits two pore calcium channel 1 (TPC1) and TPC2, is located on the lysosomal membrane, and it has recently been shown to interact with mTORC1 and to participate in nutrient sensing[106]. The nature of the interaction between lysoNa$_{ATP}$ and mTORC1 is unknown but seems to be independent form other components of the LYNUS machinery as well as TFEB and its interacting proteins (see main text).

Thus, a complex signalling machinery, which involves mTORC1 as well as additional protein complexes, is located on the lysosomal surface. This machinery, herein referred to as LYNUS (lysosome nutrient sensing), responds to the lysosomal amino acid content and signals the information both to the cytoplasm and the nucleus. The main components of the LYNUS machinery are illustrated in FIG. 2.

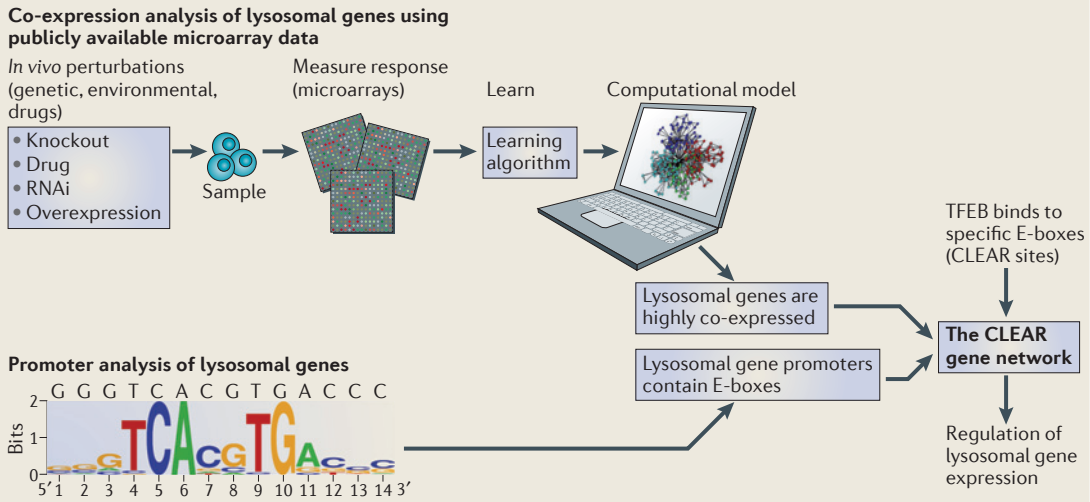
The involvement of the lysosome in nutrient sensing is a new concept that expands our view of this organelle from simply being an effector of cellular clearance to being a sensor and regulator of various cellular functions, including cell cycle progression, growth, macromolecule biosynthesis and autophagy[107]. The recent discovery of a starvation-induced lysosome-to-nucleus signalling mechanism (see below) further supports this concept[108]. Interestingly, autophagic lysosomal reformation (ALR), a recently described evolutionarily conserved process by which nascent lysosomes are formed from autolysosomal membranes, also requires mTORC1 reactivation during prolonged starvation[105,109,110]. Furthermore, prolonged starvation also controls lysosomal reformation through the kinase activity of phosphatidylinositol 4-kinase IIIβ (PI4KIIIβ)[21].

Regulation of lysosome function

The recent discovery of a 'lysosomal gene network' and of its master regulator TFEB has revealed that lysosomal function can be coordinated to respond and adapt to environmental cues. The central role of TFEB in regulating lysosomal biogenesis, lysosome-to-nucleus signalling and lipid catabolism is discussed below.

TFEB regulates lysosomal biogenesis and cellular clearance. Lysosome-mediated cellular clearance processes require the concerted action of hydrolases, the acidification machinery and membrane proteins. The expression and activity of these components must be coordinated

Box 2 | **The identification of the CLEAR gene network**



Gene networks control several aspects of cellular function and metabolism, such as the coordination of the cellular response to environmental conditions. In specialized organelles, this coordination is facilitated by compartmentalization. A systems biology approach was used to test the hypothesis that lysosomal genes are co-expressed, regulated by common factors and able to respond to similar environmental cues (see the figure). The expression behaviour of genes encoding lysosomal proteins was analysed using publicly available microarray data. This analysis revealed that lysosomal genes have a statistically significant tendency to be co-expressed in various different tissues and cell types as well as under different conditions[12]. Subsequently, pattern discovery analysis revealed the presence of a palindromic 10-base site in the promoters of known lysosomal genes. This sequence was previously identified as a specific version of a known target site for basic-helix–loop–helix (bHLH) transcription factors, also known as an E-box. Thus, these two independent approaches, namely co-expression and promoter analyses, identified a new gene network which was named CLEAR (coordinated lysosomal expression and regulation). Further studies demonstrated that transcription factor EB (TFEB), which belongs to the MITF (microphthalmia-associated transcription factor) subfamily of bHLH transcription factors, binds to CLEAR target sites in the promoters of lysosomal genes and positively regulates their expression, acting as a master regulator of the CLEAR network[12].

Bits, universal measurement of sequence conservation.

to allow optimal lysosomal function in different physiological and pathological conditions, such as growth, starvation, infection and the pathological accumulation of lysosomal substrates. This concept of lysosomal adaptation has emerged only recently, as little attention was given to the study of the transcriptional regulation of the genes encoding lysosomal proteins. The recent discovery of a lysosomal gene network — the coordinated lysosomal expression and regulation (CLEAR) network — and of its master regulator TFEB (a member of the MITF (microphthalmia-associated transcription factor) subfamily of transcription factors[111] that was previously implicated in a specific type of chromosomal translocation associated with renal carcinoma[112]) provides experimental evidence that lysosomal function is globally controlled[12]. The systems biology approach used to identify the CLEAR network is summarized in BOX 2. Consistent with its role as a modulator of the CLEAR network, TFEB positively regulates the expression of lysosomal genes, controls the number of lysosomes and promotes the ability of cells to degrade lysosomal substrates[12,113]. Further unbiased genomic and expression analyses, together with deep sequencing of TFEB chromatin-immunoprecipitates, allowed a more detailed analysis of the CLEAR network and revealed that TFEB is a central regulator of cellular degradative pathways[114]. Specifically, it activates the transcription of genes that encode proteins involved in several aspects of cellular clearance, such as lysosomal biogenesis, autophagy, exocytosis, endocytosis and additional lysosome-associated processes, such as phagocytosis, the immune response and lipid catabolism. Interestingly, many non-lysosomal proteins involved in the degradation of known autophagy substrates were also found to be members of this network[114]. These observations suggested that TFEB also regulates autophagy[114]. Indeed, TFEB overexpression in cultured cells significantly increased the number of autophagosomes and enhanced lysosome–autophagosome fusion and the degradation of long-lived proteins that are known autophagy substrates[13]. Consistently, viral-mediated overexpression of TFEB in the liver induced autophagy[13]. Thus, although the delivery of autophagy substrates to the lysosome and their degradation by lysosomal enzymes are distinct cellular processes, they are mechanistically linked by a common transcriptional regulatory programme[13,115].

Other examples of transcription factors that regulate autophagy have been reported[116–123]. The forkhead box O (FOXO) transcription factor family (including FOXO1, FOXO3, FOXO4 and FOXO6) is negatively regulated by the insulin pathway in an AKT-dependent manner. FOXO proteins are well conserved and have

a crucial role in many cellular processes, including in the regulation of autophagy[120,124]. FOXO3 is activated during fasting and mediates the transcription of many genes that directly and indirectly regulate autophagy induction[121,122]. Thus, FOXO3 regulation and function are very similar to those of TFEB, suggesting possible interactions between the two pathways. Indeed, FOXO3A overexpression increases cellular Gln levels and inhibits mTORC1 activity, leading to TFEB activation and the coordinated transcriptional activation of lysosomal biogenesis and autophagy[119]. Another transcription factor that regulates autophagy is ZKSCAN3, which belongs to the family of zinc-finger transcription factors that contain KRAB and SCAN domains and has recently been identified as a repressor of autophagy[123]. When ZKSCAN3 is silenced, cellular senescence and autophagy are promoted. By contrast, when ZKSCAN3 is overexpressed, autophagy is suppressed in diverse cellular models. ZKSCAN3 was also shown to negatively regulate the expression of genes involved in autophagy and lysosome biogenesis and function. Interestingly, starvation induces the cytoplasmic accumulation of ZKSCAN3 and thereby inhibits its activity. Conversely, nutrient availability promotes ZKSCAN3 nuclear translocation in an mTOR-dependent manner[123]. In conclusion, it seems that TFEB and ZKSCAN3 have opposing functions in regulating lysosome biogenesis and autophagy in response to cellular needs. It will be interesting to determine whether these two transcription factors work in conjunction with each other.

TFEB conveys signals from the lysosome to the nucleus. Transcriptional mechanisms that control crucial cellular functions should respond to environmental cues. Under basal conditions in most cell types, TFEB is located in the cytoplasm. However, under specific conditions, such as starvation or lysosomal dysfunction, TFEB rapidly translocates to the nucleus[12,13]. This process is controlled by the TFEB phosphorylation status; phosphorylated TFEB is located predominantly in the cytoplasm, whereas the dephosphorylated form is found in the nucleus[13]. Phosphoproteomic studies identified at least ten different phosphorylation sites in the TFEB protein, suggesting a complex regulatory mechanism[125]. At least three different kinases have been shown to phosphorylate TFEB: ERK2 (REFS 13,126); mTORC1 (REFS 108,126–129), and protein kinase Cβ (PKCβ)[95]. Phosphorylation of Ser142 by ERK2 and of both Ser142 and Ser211 by mTORC1 is crucial in determining the subcellular localization of TFEB. Mutation of either or both of these Ser residues to Ala results in the constitutive nuclear localization of TFEB[13,108,127,129]. By contrast, during osteoclast differentiation, PKCβ-induced phosphorylation of three Ser residues located in the final 15 carboxy-terminal amino acids of TFEB stabilizes the protein and increases its activity[95].

Interestingly, cytoplasmic TFEB is located both in the cytosol and on the lysosomal surface, where it interacts with mTORC1 and the LYNUS machinery[108,130] (FIG. 2). This observation suggests a mechanism by which the lysosome regulates its own biogenesis by controlling TFEB subcellular localization. Cellular conditions that lead to mTORC1 inactivation, such as stress, starvation and lysosomal inhibition, induce TFEB nuclear translocation and thus activate the lysosomal system[108,127,129]. In addition, several isoforms of the 14-3-3 protein family have an important role in controlling TFEB subcellular localization by retaining phosphorylated TFEB in the cytoplasm[127,129]. More recently, TFEB was shown to interact with active RAG GTPases[130]. This interaction promotes the lysosomal localization of TFEB and its mTORC1-dependent phosphorylation[130]. Interestingly, other members of the basic-helix–loop–helix (bHLH) family of transcription factors, such as MITF and TFE3, the sequences of which are closely related to TFEB, seem to be regulated by a similar mechanism[129,130]. It will be interesting to investigate whether other additional mTOR-independent mechanisms also regulate the nuclear translocation of TFEB.

Recent data indicate that cellular nutrient levels regulate TFEB also at the transcriptional level. The absence of serum and amino acids from the cell culture medium induces TFEB expression, whereas their re-addition inactivates this transcription factor. Similarly, depriving mice of food for 24 hours induces TFEB expression in multiple tissues[131]. Interestingly, the transcriptional response of TFEB to nutrients is mediated by an autoregulatory feedback loop in which TFEB binds to its own promoter in a starvation-dependent manner and induces its own expression[131]. Thus, the regulation of TFEB activity by nutrients involves a rapid, phosphorylation-dependent post-transcriptional switch, which is responsible for the nuclear translocation of TFEB, and a transcriptional autoregulatory component, which allows for a slower, more sustained response. This complex regulation mediates the cellular starvation response by inducing lipid catabolism[131] (see below).

In conclusion, TFEB participates in a lysosome-to-nucleus signalling mechanism that conveys information on the lysosomal status to the nucleus to trigger a transcriptional response. This crosstalk between the lysosome and the nucleus controls cellular clearance and energy metabolism. A proposed model of TFEB regulation by nutrients is illustrated in FIG. 2.

TFEB regulates lipid catabolism. Autophagy has a central role in lipid metabolism by shuttling lipid droplets to lysosomes, where they are hydrolysed into free fatty acids (FFAs) and glycerol. This process, called macrolipophagy[132,133], indicates the presence of a tight relationship between intracellular lipid metabolism and lysosomes. Interestingly, excessive lipid overload may in turn inhibit autophagy. This could be caused by either an alteration of the composition of the lysosomal membrane, rendering it less prone to fusion with autophagosomes[134,135], or by the downregulation of autophagy genes[136]. Restoring liver autophagy ameliorates the metabolic phenotype of genetically induced obese mice (*Ob/Ob*), suggesting that enhancing lysosomal function may be a possible therapeutic strategy for the treatment of obesity[136]. Interestingly, lysosomal dysfunction was associated with an altered energy balance in mouse models of LSDs[137]. In addition, in Wolman's disease, the

Wolman's disease
An early-onset fulminant disorder of infancy with substantial infiltration of several organs, including the spleen and the liver, by macrophages filled with cholesteryl esters and triglycerides. It is caused by mutations in the gene encoding lipase A.

deficiency of lysosomal acid lipase leads to a severe intracellular fat accumulation[138].

These studies suggest that the regulation of the lysosomal and autophagic pathways may have an effect on cellular lipid metabolism. Indeed, TFEB was found to regulate liver lipid metabolism[131]. Transcriptome analysis in the mouse liver after viral-mediated TFEB overexpression revealed that this transcription factor positively regulates the expression of genes involved in several steps of lipid breakdown, such as lipophagy, fatty acid oxidation and ketogenesis. Interestingly, *PPARα* (peroxisome proliferator-activated receptor-α) and *PGC1α* (PPARγ co-activator 1α), which are key regulators of lipid metabolism in response to starvation[131,139], are significantly induced by TFEB. In addition, TFEB was shown to directly bind to the *PGC1α* promoter in a starvation-sensitive manner[131].

Remarkably, whereas liver-specific TFEB knockout caused defective lipid degradation during starvation, TFEB overexpression enhanced liver fat catabolism and prevented diet-induced obesity[131]. Thus, TFEB controls the cellular response to nutrient levels and induces a metabolic switch that allows the organism to generate energy from stored lipids. These observations shed new light on the role of the lysosome in cellular energy metabolism and the mechanisms underlying obesity and metabolic syndrome. A proposed model for the role of TFEB in lipid catabolism is illustrated in FIG. 2.

TFEB regulation and function are evolutionarily conserved. The *Caenorhabditis elegans* genome encodes a single homologue of TFEB, HLH-30, which is a transcription factor that recognizes a DNA motif similar to the CLEAR motif and drives the transcription of metabolic genes[140]. HLH-30 acts in a similar manner to TFEB during *C. elegans* starvation. *Hlh-30* mRNA progressively accumulates during starvation and rapidly decreases after the re-addition of food, as is the case with mammalian TFEB[131,141]. HLH-30 also responds to starvation in a manner similar to its human counterpart, as it can be detected mainly in the cytoplasm of well-fed *C. elegans* and predominantly in the nucleus of fasting animals[141]. The autoregulatory loop that regulates TFEB transcription is conserved in *C. elegans*[131]. Interestingly, HLH-30 activity is required to mobilize cytosolic lipids in fasting nematodes. Starved *hlh-30* mutants failed to mobilize lipids as promptly as wild-type animals[131,141], indicating that HLH-30 is required for *C. elegans* to efficiently use lipid stores during periods of starvation. HLH-30 is essential for the induction of lipid catabolism genes such as *lipase 2*, *lipase 3* and *lipase 5* during fasting[141], and the induction of these genes is greatly compromised in starved nematodes when *hlh-30* is deleted[131]. Notably, starved *hlh-30* mutants fail to mobilize their lipid stores due to a severe transcriptional response defect.

In wild-type *C. elegans,* starvation results in lifespan extension[142]. However, loss of *hlh-30* was shown to result in the abrogation of starvation-induced lifespan extension[131,141], suggesting an important role for HLH-30 (and possibly TFEB) in longevity. Consistent with this, nematodes that are mutant for *daf-2*, which encodes the insulin-like growth factor 1 (IGF-1) receptor, have an increased lifespan, and it has been shown that they exhibit upregulated autophagy [143]. In conclusion, HLH-30 and mouse TFEB share evolutionarily conserved functions in the adaptation of organisms to starvation. As observed for mammalian TFEB, *hlh-30* expression is autoregulated, required for lipid mobilization and is essential for the starvation response. The *C. elegans* model will be very useful for studying, in more detail, the potential role of TFEB in cell survival and ageing in different conditions, considering that TFEB total loss of function is embryonic lethal in mice[144]. The striking conservation of TFEB function in *C. elegans* suggests that this regulatory mechanism evolved early to facilitate organismal adaptation to challenging nutritional conditions[131,141].

Lysosomal dysfunction and human disease

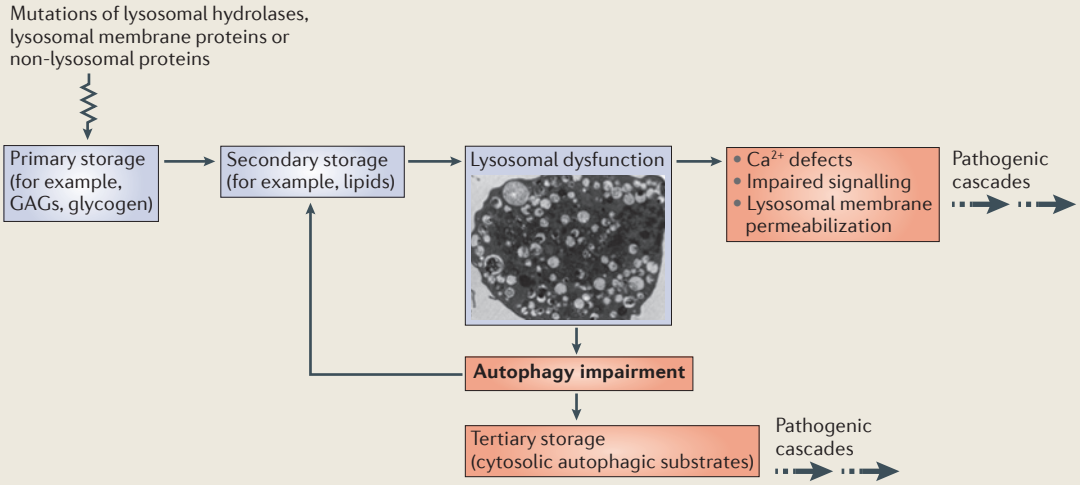
Lysosomal dysfunction has been associated with several human diseases, as well as with the process of ageing, which may be associated with a decline in lysosomal function and a progressive accumulation of intracellular material (for example, lipofuscin and ubiquitin)[145]. Indeed, enhancement of the autophagic—lysosomal pathway seems to be an important determinant of the anti-ageing effect of caloric restriction[146]. The identification of factors that regulate lysosomal biogenesis and function, such as TFEB, should pave the way to the development of novel therapeutics for diseases in which lysosomal dysfunction is aberrant.

Lysosomal dysfunction in LSDs and neurodegenerative diseases. For more than three centuries, it has been known that genetic defects in specific lysosomal components lead to the accumulation of substrates that are not degraded in the lysosomal lumen, followed by progressive lysosomal dysfunction in several tissues and organs. These disorders are known as LSDs. The classification of LSDs and their clinical features has been reviewed in detail in several recent articles[147–152]. Although these diseases were among the first for which both the biochemical and the molecular basis were recognized, the mechanisms by which the storage of material that has not been degraded in lysosomes translates into cellular and tissue dysfunction and clinical symptoms have yet to be fully elucidated. The main mechanisms that have been identified so far are summarized in BOX 3. A global impairment of lysosomal function has an important role in the pathogenesis of several LSDs, because a deficiency in individual lysosomal proteins can have broad consequences on the basic functions of lysosomes[147]. In particular, several studies have demonstrated an impairment of the autophagic pathway in LSDs[147,153–157]. This results in the secondary accumulation of autophagy substrates, such as dysfunctional mitochondria and polyubiquitylated proteins, which have a crucial role in disease pathogenesis[157]. A block of autophagy in LSDs may be caused by a defect in the fusion between lysosomes and autophagosomes, as observed in multiple sulphatase deficiency (MSD) and mucopolysaccharidosis type IIIA (MPSIIIA), which may be caused by abnormalities in membrane lipid composition and SNARE protein distribution[156].

Multiple sulphatase deficiency
(MSD). An autosomal recessive inherited disease that is caused by mutations in the sulphatase-modifying factor 1 (*SUMF1*) gene.

Mucopolysaccharidosis
(MPS). A metabolic disorder that is caused by the absence or malfunctioning of lysosomal enzymes needed to break down molecules.

Box 3 | **Mechanisms of lysosomal storage diseases (LSDs)**



LSDs are a group of rare and recessively inherited metabolic dysfunctions with an overall incidence of 1 in 5000. LSDs are caused by mutations of genes encoding proteins that localize to the lysosomal lumen, lysosomal membrane or other cellular compartments that contribute to lysosomal function. These disorders are characterized by the progressive accumulation of material that has not been degraded in the lysosomes of most cells and tissues. Approximately 60 different types of LSDs have been recognized. Historically, LSDs have been classified on the basis of the type of material that accumulates in the lysosomes, such as mucopolysaccharides, sphingolipids, glycoproteins, glycogen and lipofuscins. LSDs often show a multisystemic phenotype that is associated with severe neurodegeneration, mental decline, cognitive problems and behavioural abnormalities. Other tissues that are commonly affected are bone and muscle. Cell and tissue pathology are the result of a complex series of pathogenic cascades that occur downstream of lysosomal dysfunction. The figure illustrates the main steps underlying LSD pathogenesis. Mutations in genes that are important for lysosomal function result in the accumulation of specific substrates that have not been degraded in the lysosome (primary storage). This leads to the accumulation of additional lysosomal substrates (secondary storage) due to a blockage in lysosomal trafficking. Excessive lysosomal storage has a broad impact on lysosomal function by causing defects in Ca^{2+} homeostasis, signalling abnormalities and lysosomal membrane permeabilization. In addition, lysosomal dysfunction is associated with autophagy impairment, due to defective fusion between lysosomes and autophagosomes. This causes the accumulation of autophagic substrates such as aggregate-prone proteins and dysfunctional mitochondria (tertiary storage), which contributes to neurodegeneration. GAGs, glycosaminoglycans.

Current therapeutic strategies for LSDs are aimed at either restoring or replacing the activity of defective lysosomal enzymes, and they involve molecular chaperones, enzyme replacement or viral-mediated gene therapy[158]. Inhibition of substrate synthesis is another available therapeutic option for some LSDs[158]. These strategies, however, have limitations, such as the difficulty of targeting the enzyme, or the gene, to the required sites in the body. For instance, a major hurdle for delivering therapies to the brain is the difficulty that these enzymes encounter in crossing the blood–brain barrier. Considering that LSDs include over 60 different diseases and that in most cases each therapy is strictly disease-specific, the overall costs of preclinical studies and clinical trials will be extremely high.

Accumulating evidence indicates that lysosomal and autophagy dysfunction is one of the main mechanisms underlying common neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and Huntington's disease[159,160] (FIG. 3). Mutated aggregate-prone proteins that cause neurodegenerative diseases, such as expanded huntingtin (HTT) in Huntington's disease and mutated α-synuclein in Parkinson's disease, are cleared by boosting the lysosomal–autophagic pathway[161–163]. In addition, aggregate-prone proteins may in turn affect the efficiency of autophagy by inhibiting cargo recognition by autophagosomes[164,165].

Mutations in genes encoding essential components of the endolysosomal—autophagic pathway have also been described in several neurodegenerative diseases. A significant number of patients with Parkinson's disease, particularly among Ashkenazi Jews[166], are heterozygous for mutations in the gene encoding the lysosomal enzyme β-glucocerebrosidase[167]. Homozygous mutations in the same gene cause Gaucher's disease, a neurodegenerative LSD[168]. It has been proposed that lower levels of β-glucocerebrosidase lead to an increased accumulation of glucosylceramide in the lysosome, and this in turn accelerates the synthesis and stabilization of soluble α-synuclein oligomers that eventually convert into amyloid fibrils. Furthermore, the accumulation of α-synuclein also blocks the trafficking of newly synthesized β-glucocerebrosidase to the lysosome and thus further amplifies glucosylceramide accumulation[169]. In addition, mutations in ATPase type 13A2 (*ATP13A2*), a component of the lysosomal acidification machinery, were found in patients with hereditary parkinsonism[170] and are associated with lysosomal dysfunction, defective

Gaucher's disease
An autosomal recessive lysosomal storage disorder due to the deficient activity of β-glucocerebrosidase.

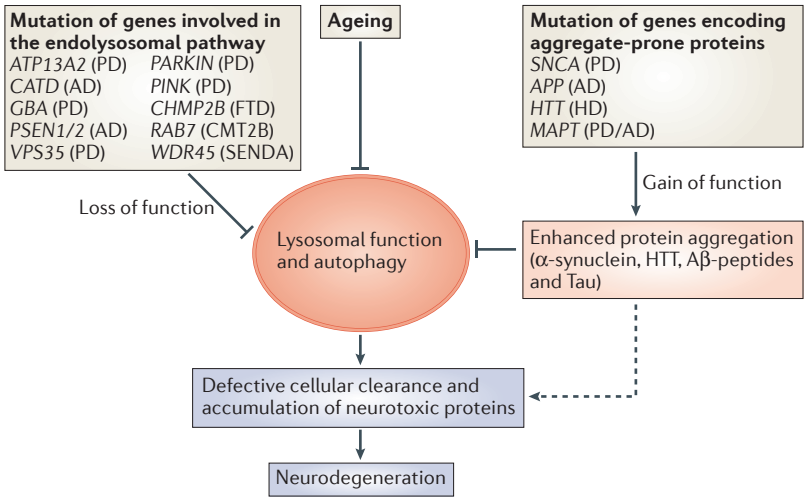


Figure 3 | **Defective cellular clearance in neurodegenerative diseases.** Defective cellular clearance, leading to neurodegeneration, can result from two different mechanisms. First, loss-of-function mutations of genes involved in the lysosomal–autophagic pathway (for example, *ATP13A2* (ATPase type 13A2), *CATD* (cathepsin D), *GBA* (beta-glucosidase, acid), *PSEN1* (presenilin 1), *PSEN2*, *VPS35* (vacuolar protein sorting 35), *PARKIN* (Parkinson's disease protein), *PINK* (PTEN-induced putative kinase), *CHMP2B* (charged multivesicular body protein 2B), *RAB7* and *WDR45* (WD repeat 45)) can affect cellular degradation and recycling processes. Second, gain-of-function mutations of aggregate-prone proteins (for example, *SNCA* (alpha-synuclein), *APP* (amyloid precursor protein), *HTT* (huntingtin) and *MAPT* (microtubule-associated protein TAU) may lead to enhanced protein aggregation and impairment of lysosomal–autophagic pathways. In addition, a global decrease of lysosomal–autophagy function has been observed during ageing and may contribute to an impairment of cellular clearance. Ultimately, and regardless of the mechanism involved, defective cellular clearance leads to the accumulation of neurotoxic proteins and neuronal cell death. AD, Alzheimer's disease; CMT2B, Charcot–Marie–Tooth type 2B; FTD, fronto-temporal dementia; PD, Parkinson's disease; SENDA, static encephalopathy of childhood with neurodegeneration in adulthood.

Fronto-temporal dementia
A disorder associated with fronto-temporal lobar degeneration.

Charcot–Marie–Tooth type 2B
Autosomal dominant peripheral sensory neuropathy due to mutations in the late endosomal small GTPase RAB7.

Neuronal ceroid lipofuscinosis
A clinically and genetically heterogeneous group of neurodegenerative disorders that are characterized by the intracellular accumulation of autofluorescent lipopigment storage material.

Pompe's disease
An autosomal recessive inherited disease, also known as glycogen storage disease II. This prototypic lysosomal storage disease is caused by mutations in the gene encoding acid α-1,4-glucosidase.

clearance of autophagosomes and accumulation of α-synuclein[171]. Similarly, mutations in the genes encoding PINK (PTEN-induced putative kinase) and PARKIN (Parkinson's disease protein) are associated with the defective clearance of mitochondria via an organelle-specific type of autophagy known as mitophagy, leading to Parkinson's disease[172–175]. Parkinson's disease was also observed in patients carrying mutations in the *VPS35* (vacuolar protein sorting 35), which encodes an endosomal protein involved in the retrograde transport between endosomes and the TGN[176,177].

Lysosomal and autophagy dysfunction have also been identified in patients with Alzheimer's disease carrying mutations in presenilin 1 (*PSEN1*)[178]. At least two different mechanisms, one involving a defect in lysosomal acidification machinery[178] and the other a defect in lysosomal Ca^{+2} homeostasis[179], have been proposed to explain lysosomal dysfunction in these patients. Additional examples of neurodegenerative diseases that are caused by mutations of proteins involved in endosome and lysosome maturation include fronto-temporal dementia and Charcot–Marie–Tooth type 2B, which are due to mutations in charged multivesicular body protein 2B (CHMP2B)[180] and RAB7 (REF. 181), respectively. Of note, a mutation in the autophagic protein WD repeat domain 45 (WDR45) has been recently associated with SENDA (static encephalopathy of childhood with neurodegeneration in adulthood), a neurodegenerative disease characterized by iron accumulation in the brain[182].

TFEB activation as a potential therapy. The similarities between the mechanisms that lead to LSDs and common neurodegenerative diseases suggest that therapeutic strategies aimed at rescuing and/or enhancing lysosomal and autophagic function may have an impact on both types of diseases. Several attempts have been made to treat animal models of neurodegenerative diseases by enhancing the lysosomal—autophagic pathway[160,183–189]. An appealing therapeutic perspective, which has become available since the recent discovery of TFEB, would be to enhance cellular clearance by inducing TFEB function. Preliminary evidence showed that cells with enhanced TFEB levels displayed a faster rate of glycosaminoglycans (GAGs) clearance compared with control cells[12]. TFEB overexpression also resulted in a striking reduction of GAGs and cellular vacuolization in glia-differentiated neuronal stem cells (NSCs) that were isolated from mouse models of MSD and MPSIIIA, two severe types of LSD[86]. Similar results were obtained using this approach in cells from patients with other types of LSDs and/or the respective mouse models, including neuronal ceroid lipofuscinosis type 3 (due to mutations of the *CLN3* gene; also known as Batten disease) and Pompe's disease[86]. In all cases, TFEB overexpression led to the clearance of the storage material within cells. TFEB-mediated cellular clearance was also observed *in vivo* in mouse models of MSD and Pompe's disease upon viral-mediated *TFEB* gene transfer[86,190]. TFEB overexpression in a mouse model of Pompe's disease reduced glycogen load and lysosomal size, improved autophagosome processing and alleviated the accumulation of autophagic vacuoles in muscle from affected mice. Interestingly, the clearance effect of TFEB was found to be dependent on the autophagy pathway, and in the muscle, TFEB was shown to induce exocytosis of autophagolysosomes (also known as autolysosomes) via their fusion with the plasma membrane[190]. Notably, TFEB was also used as a tool to promote cellular clearance in common neurodegenerative diseases. *TFEB* gene delivery in a mouse model of Parkinson's disease ameliorated tissue pathology[191]. In a recent study, TFEB was identified as the main mediator of the ability of PGC1α to promote cellular clearance and to decrease neurotoxicity in a mouse model of Huntington's disease[192]. Finally, TFEB overexpression in the liver of mice carrying a mutated form of α1-antitrypsin resulted in the clearance of this mutated protein and rescued the liver fibrosis phenotype[193].

The mechanism by which TFEB promotes the clearance of storage material needs to be further elucidated. TFEB induction rescues lysosomal storage in LSDs in spite of a complete deficiency of one or more lysosomal enzymes. A prevailing mechanism in this case may be the activation of lysosomal exocytosis, by which the stored material may be secreted from cells upon TFEB overexpression. However, in general, it is possible that TFEB-mediated cellular clearance is the result of the combined effects of lysosomal biogenesis, autophagy and lysosomal exocytosis (FIG. 4). The possibility of pharmacologically

modulating lysosomal function, for example by inhibiting TFEB phosphorylation or by increasing TFEB dephosphorylation, represents an attractive therapeutic strategy to promote cellular clearance in all of the above-mentioned diseases. Therefore, drug screening approaches aimed at identifying molecules that promote TFEB nuclear translocation present an interesting path forward. However, careful, long-term studies evaluating the potential side effects will be required. Pulsatile treatments that can increase TFEB activity for only limited periods of time may be the best option for diseases in which the accumulation of storage material takes a long time. At this stage, it is too early to determine whether TFEB induction will be a viable therapeutic option for LSDs or for other diseases. However, the broad range of diseases that might be treated by this therapeutic strategy make it a very appealing avenue.

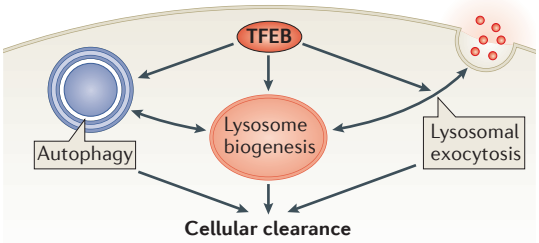


Figure 4 | **TFEB regulates cellular clearance.** Transcription factor EB (TFEB) controls lysosomal biogenesis by regulating the level of lysosomal enzymes, lysosomal acidification and the number of lysosomes. TFEB also controls autophagy by regulating the number of autophagosomes and the fusion between autophagosomes and lysosomes. Finally, TFEB regulates the docking and fusion of lysosomes to the plasma membrane in the process of lysosomal exocytosis. The concerted action of these three processes leads to cellular clearance.

Conclusions and future perspectives

The emerging role of the lysosome in important processes, such as nutrient sensing, signalling and metabolism, requires further investigation — what we know today is just the 'tip of the iceberg'. Systematic approaches, such as transcriptomics, proteomics and metabolomics, coupled with the power of systems biology, will be particularly important for identifying all the components of the lysosome and understanding the role of the greater lysosomal system[152]. These approaches should be complemented by *in vivo* imaging and intravital microscopy, which allow the visualization of lysosomes in the context of a living organism and in specific physiological or pathological conditions.

Interdisciplinary approaches will also allow us to answer intriguing questions. How does lysosome number, size and content vary in different cell types, in different tissues or in different individuals? Do different types of lysozymes exist with specialized roles? To what extent do environmental or pathological conditions influence the composition, function or identity of lysosomes? What is the physiological role of lysosomal signalling and its involvement in human disease?

Besides the involvement in neurodegenerative diseases, the role of the lysosome in other pathological processes, such as abnormalities of lipid metabolism, infections and even ageing, is still largely unexplored. Transcriptomic and proteomic analysis of patient-derived tissues and whole genome and exome sequencing of the DNA of patients may lead to the discovery of lysosomal variation as a predisposing factor for additional human diseases. Furthermore, studying lysosomal function in various disease processes will affect the development of novel therapeutic strategies. Moreover, the development of high-content screening approaches will pave the way for the identification of novel compounds that are able to modulate lysosomal function, which could in turn be used as effective drugs to promote cellular clearance.

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