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

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Preface

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

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-bilayer lipid 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 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 through the endocytic pathway[3], while 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, known as lysosomal “exocytosis”, is very active in particular cell types, such as cells from the hematopoietic lineage[9], osteoclasts[10] and melanocytes[11]. In addition to cellular clearance and secretion, the

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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, and 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 to 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 will first describe the structure of the lysosome and its established role in **cellular clearance**. We will then consider the emerging roles of lysosomes, including their function in **plasma membrane repair** and **signaling**, before discussing the identification of the transcription factor EB (TFEB) as a key molecule that regulates lysosomal biogenesis and autophagy[12, 13]. Finally, we will discuss how lysosomal dysfunction leads to human disease.

Lysosome structure

The complex series of events leading to the formation of a mature lysosome have 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 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, 23, 24] and N-ethylmaleimide-sensitive attachment protein (SNAP) receptor (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 GTPase and SNAREs[28]. RAB5 and RAB7 are specifically involved in the tethering and docking processes during endo-lysosomal membrane trafficking pathways[23, 24]. Furthermore, a reduction in RAB5 levels results in a decreased number of endosomes and lysosomes and in a block of the endocytic pathways[29]. A specific combinatorial set of SNAREs, including VAMP7, VAMP8, VTI1B, syntaxin7 and syntaxin8, form 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 endo-lysosomal 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 sulfatases, glycosidases, peptidases, phosphatases, lipases, and nucleases, which allow the lysosome to hydrolyze a vast repertoire of biological substrates, ranging from glycosaminoglycans and sphingolipids to 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 uptake lysosomal enzymes via the mannose-6-phosphate receptor 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 LIMP-2, was recently identified for β-glucocerebrosidase[34].

The selective degradation of intra-lumenal membranes and lipids within lysosomes occurs in specialized intra-lysosomal vesicles that 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]. The study of patients with defects of glycosphingolipid catabolism was instrumental for the understanding of this complex process [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 mannose 6 phosphate receptors, CI-MPR and 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], and SUMF1, an ER-resident protein responsible for a post-translational modification, a conversion of highly conserved cysteine in the active site to alpha-formyl-glycine, which is required for the activation of all sulfatases[43, 44].

A variety of methods have been used to purify lysosomes and analyze their proteome[45-49]. Some of these approaches are based on subcellular fractionations, while 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 lysosomal 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 resident proteins. Based on current data, a little over 100 *bona fide* lysosomal resident proteins have been identified; approximately 70 of these are lysosomal matrix proteins and approximately 50 are lysosomal membrane proteins[48]. However, these numbers are likely to grow in the near future.

Lysosome functions

Lysosomal functions can be schematically divided into three main types: degradation, secretion and signaling (Figure 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 endocytosis 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]. Signaling 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 signaling[54-56].

A known hallmark of endosome-to-lysosome maturation is the progressive decrease of the internal pH to around pH 5 in the mature lysosome[57]. This is crucial for the release of acid hydrolases from mannose-6-phosphate receptors 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 ClC-7[59-61] and the cation transporters MCOLN1 and TPC2[62], which mediate Ca^{2+} release from the lysosome[63-65]. 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 into 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 through the LAMP2a protein[5, 16, 68, 69]. Macroautophagy, herein referred to as autophagy, relies on the biogenesis of autophagosomes, 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 is able to degrade 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]. Originally lysosomal exocytosis was thought to be limited to professional secretory cells containing 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 defense 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 v- SNARE VAMP-7 and synaptotagmin VII (SytVII) on lysosomes, and the 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 mucolipin 1 (MCOLN1, TRPML1)[85-87]. It was also postulated that autophagy proteins may regulate lysosomal exocytosis. For example, lipidation of the late autophagosome marker protein 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 per se may not be mediating this process[89].

Lysosomal exocytosis is not only responsible for the secretion of lysosomal content, but it plays a crucial role in **plasma membrane repair**. Plasma membrane injuries induce a 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 important also in defense mechanisms against bacterial infection[93], and has been implicated in a specific type of muscular dystrophy, which is characterized by a defect in muscle fiber repair[94].

Lysosomal exocytosis is transcriptionally regulated by TFEB, a master gene for 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 mucolipin 1-mediated elevation of intracellular Ca^{2+} [86]. **Interestingly, TFEB mediated regulation of lysosomal exocytosis plays an important role in osteoclast differentiation and bone resorption**[95].

Signaling from lysosomes

It has become evident that the lysosome plays an important role in nutrient sensing and in signaling pathways that are involved in cell metabolism and growth. Remarkably, the **mammalian target of rapamycin complex 1 (mTORC1)** kinase complex, 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, stress and

oxygen are the major activators of mTORC1, which in turn positively regulates protein, mRNA and 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/mammalian autophagy-related gene 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 activation of ULK1–ATG13–FIP200 and autophagy. Thus, the level of cellular autophagy is inversely correlated with mTORC1 activity, and the pharmacological inhibition of mTORC1 potently stimulates autophagy.

Recent studies showed that the level of amino acids inside the lysosome 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 dock and become activated[104]. This observation supports 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 showed that an ATP-sensitive Na^+ 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 the lysoNa$_{ATP}$ channel becomes constitutively open. Thus, lysoNa$_{ATP}$ regulates lysosomal pH stability and amino acid homeostasis by responding to ATP levels and controlling lysosomal membrane potential[106]. Thus, a complex signaling 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 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 Figure 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 a variety of cellular functions, ranging from cell cycle progression and growth to macromolecule biosynthesis and autophagy[107]. The recent discovery of a starvation-induced lysosome-to-nucleus signaling 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 PI4KIIIβ and important regulator of lysosomal efflux [21]

Regulation of lysosome function

The recent discovery of a “lysosomal gene network” and of its master gene TFEB, has revealed that lysosomal function can be coordinated to respond and adapt to environmental cues. Here we discuss the central role of TFEB in regulating lysosomal biogenesis, lysosome-nucleus signalling and lipid catabolism.

TFEB regulates lysosomal biogenesis and cellular clearance

Lysosome-mediated cellular clearance processes require the concerted action of hydrolases, acidification machinery and membrane proteins. The expression and activity of these components must be coordinated to allow optimal lysosomal function in different physiological and pathological conditions, such as growth, starvation, infection, and the intracellular accumulation of storage products. This concept of **lysosomal adaptation** has only emerged 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 subfamily of transcription factors[111] that was previously implicated in a 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 summarised 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, integrated with deep sequencing of TFEB chromatin-immunoprecipitates, provided 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 the network[114]. These observations suggested that TFEB also regulates autophagy[114]. Indeed, TFEB overexpression in cultured cells significantly increases the number of autophagosomes, and enhances lysosome-to-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 regulation[13, 115].

Other examples of transcription factors-regulating autophagy have been reported[116-123]. The FOXO transcription factor family (FOXO1, 3, 4 and 6) is negatively regulated by the insulin pathway in an AKT dependent manner. FOXOs are well conserved and have a critical 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]. FOXO3 regulation and function is very similar to that of TFEB, suggesting possible interactions between the two pathways. Indeed, FOXO3A overexpression increases cellular glutamine levels and inhibits mTORC1 activity, leading to TFEB activation and resulting in the coordinated transcriptional activation of lysosomal biogenesis and autophagy[119] Another transcription factor regulating autophagy is the recently described 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. 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 inhibiting its activity. Conversely, nutrient availability promotes ZKSCAN3 nuclear translocation in an mTOR-dependent manner[123]. In conclusion, it appears that TFEB and ZKSCAN3 work in opposite directions to regulate 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 from the cytoplasm to the nucleus[12, 13]. The nuclear translocation of TFEB is controlled by its **phosphorylation** status. Phosphorylated TFEB is located predominantly in the cytoplasm, while its dephosphorylated form is found in the nucleus[13]. Phosphoproteomic studies identified at least 10 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[13, 126], mTORC1[108, 126-129], PKCβ[95]. The phosphorylation of Ser142 by ERK2 and of both Ser142 and Ser211 by mTORC1, are crucial in determining the subcellular localization of TFEB. Mutation of either or both of these serines into alanines results in the constitutive nuclear localization of TFEB[13, 126-129]. On the other hand, during osteoclasts differentiation PKCβ-mediated phosphorylation of three serine residues located in the last 15 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] (Figure 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 play 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 guanosine triphosphatases (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 sequence of which is closely related to TFEB, appear 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 also regulate TFEB at the transcriptional level. The absence of serum and amino acids from the cell culture medium induces TFEB

expression, while their re-administration turns it off. In a similar manner 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 (see below)[131].

In conclusion, TFEB participates in a lysosome-to-nucleus signaling mechanism, which conveys information on lysosomal status to the nucleus to trigger a transcriptional response. This “cross-talk” between the lysosome and the nucleus controls cellular clearance and energy metabolism. A proposed model of TFEB regulation by nutrients is illustrated in Figure 2.

TFEB regulates lipid catabolism

Autophagy plays a central role in lipid metabolism by shuttling lipid droplets to lysosomes, where they are hydrolyzed 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 down-regulation 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 murine models of LSDs[137]. In addition, in Wolman disease, the 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 impact 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, *PGC1α* and *PPARα*, 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, while liver-specific TFEB knock-out (KO) caused defective lipid degradation during starvation, TFEB overexpression enhanced liver fat catabolism and prevented diet-induced obesity[131]. Thus, TFEB controls the starvation response by responding to nutrient levels, and by inducing 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 on mechanisms underlying obesity and metabolic syndrome. A proposed model for the role of TFEB in lipid catabolism is illustrated in Figure 2.

TFEB regulation and function are evolutionarily conserved

The *C. 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-introduction of food, as is the case with mammalian TFEB[131, 141]. The HLH-30 protein 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 lipid catabolism 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 HLH30 and TFEB in longevity. Consistently, worms that are mutant for daf-2, which encodes the insulin-like growth factor 1 (IGF-1) receptor in *C. elegans*, have an increased life-span and it has been shown that they upregulate autophagy[143]. In conclusion, HLH-30 and murine 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 aging 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 **aging,** 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 appears to be an important determinant of the anti-aging effect of caloric restriction[146]. The identification of genes 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 leads to the accumulation of undegraded substrates in the lysosomal lumen,

followed by progressive lysosomal dysfunction in several tissues and organs. These disorders are known as **lysosomal storage diseases (LSDs)**. The classification of LSDs, as well as 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 undegraded material in lysosomes translates into cellular and tissue dysfunction and clinical symptoms has yet to be fully elucidated. The main mechanisms that have been identified so far are summarized in Box 3. In this context, a **global impairment of lysosomal function** plays 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 poly-ubiquitinated proteins, which play 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 Sulfatase Deficiency (MSD) and Mucopolysaccharidosis type IIIA (MPS IIIA), which may be caused by abnormalities in membrane lipid composition and SNARE protein distribution[156].

Current therapeutic strategies for LSDs are aimed at either restoring or replacing the activity of defective lysosomal enzymes by the use of 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 suffer from major limitations, such as the difficulty of delivering the enzyme, or the gene, to the required target sites in the body. For instance, a major hurdle for delivering therapies to the brain is crossing the blood-brain-barrier (BBB). In addition, in most cases each therapy is strictly disease-specific. This makes the overall costs of preclinical studies and clinical trials for LSDs extremely high, when one considers that LSDs include over 60 different disease entities.

Accumulating evidence indicates that lysosomal and autophagy dysfunction is one of the main mechanisms underlying common neurodegenerative diseases such as Parkinson's (PD), Alzheimer's (AD), and Huntington's (HD) diseases[159, 160] (Figure 3). Mutated aggregate-prone proteins that cause neurodegenerative diseases, such as expanded huntingtin in HD and mutated α-synuclein in PD, 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 PD, 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 lysosomal storage disease[168]. It has been proposed that lower levels of β-glucocerebrosidase lead to an increased accumulation of glucosylceramide that 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 ATP13A2, a component of the lysosomal acidification machinery, were found in patients with hereditary Parkinsonism[170] and are associated with lysosomal dysfunction, defective clearance of autophagosomes and accumulation of α-synuclein[171]. Similarly, mutations in PINK and PARK genes are associated with the defective clearance of mitochondria via an organelle-specific type of autophagy known as mitophagy, leading to Parkinson disease [172-175]. PD was also observed in patients carrying mutations in the VPS35 gene, which encodes an endosomal protein involved in the retrograde transport between endosomes and the Trans-Golgi network [176, 177].

Lysosomal and autophagy dysfunction have also been identified in patients with AD carrying mutations of the presenilin 1 gene[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 due to 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 CHMP2B[180] and RAB7[181], respectively. Of note, a mutation in the autophagic protein WD repeat domain 45 (WDR45) has been recently associated with static encephalopathy of childhood with neurodegeneration in adulthood (SENDA), a neurodegenerative disease characterized by iron accumulation into the brain[182].

TFEB activation as a potential therapy

The similarity 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 impact 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 to controls[12]. TFEB overexpression also resulted in a striking reduction of GAGs and of 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 and/or from murine models other types of LSDs, including Neuronal Ceroid Lipofuscinosis type 3 (CLN3, Batten disease) and Pompe disease[86]. In all cases TFEB overexpression led to the clearance of the storage material within the cells. TFEB-mediated cellular clearance was also observed *in vivo* in murine models of MSD and Pompe disease upon TFEB viral-mediated gene transfer[86, 190]. TFEB overexpression in a mouse model of Pompe disease reduced glycogen load and lysosomal size, improved autophagosome processing and alleviated the accumulation of autophagic vacuoles. 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 murine model of PD 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 rescue neurotoxicity in a murine model of HD[192]. Finally, TFEB overexpression in the liver of mice carrying a mutated form of α1 anti-trypsin resulted in the clearance of the mutated protein and in the rescue of liver fibrosis[193].

The mechanism by which TFEB promotes the clearance of storage material needs further elucidation. 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 storage material may be secreted outside the cells upon TFEB overexpression. However, in general, it is likely 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 approach 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. Careful, long-term studies for the evaluation of potential side effects will have to be undertaken. In this context pulsatile treatments, in which one can boost TFEB activity only for limited periods of time, may be the best option for diseases in which storage material takes a long time to accumulate. At this stage it is too early to determine whether TFEB induction will end up being a viable therapeutic option for LSDs or for other diseases. However, the broad spectrum of diseases on which this therapeutic strategy may impact makes it a very appealing avenue.

Conclusions and future perspectives

The emerging role of the lysosome in important processes, such as nutrient sensing, signaling, and metabolism, requires further investigation - what we see 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 of the components of the lysosome and understanding the role of the “greater lysosomal system”[152]. These systematic 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 such as: how does lysosome number, size, and content vary in different cell types, in different tissues, or in different individuals? How many different types of lysosomes are there 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 signaling 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

aging, is still largely unexplored. Transcriptomic and proteomic analysis of patient-derived tissues and whole genome and exome sequencing of patients' DNA may lead to the discovery of lysosomal “variation” as a predisposing factor for additional human diseases. Furthermore, studying lysosomal function in a variety of disease processes will have a significant impact in developing novel therapeutic strategies. In this context, the development of high content screening approaches will pave the way to the identification of novel compounds that are able to modulate lysosomal function, which could in turn be made into effective drugs to promote cellular clearance.

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Glossary

Glycocalyx	The polysaccharide-based coating on the inner side of a lysosomal membrane that protects it from digestion by lysosomal enzymes
mucolipidosis IV	Mucolipidosis IV is an autosomal recessive neurodegenerative lysosomal storage disorder due to mutations in the gene encoding mucolipin-1. It is characterized by psychomotor retardation and ophthalmologic abnormalities

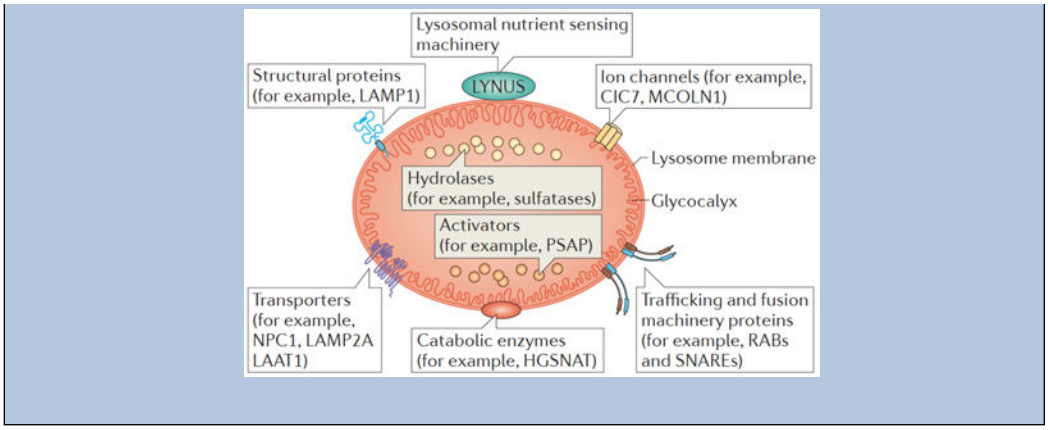
Danon disease	Danon disease is an X-linked dominant disorder due to mutations in the gene encoding lysosome-associated membrane protein-2 (LAMP2), predominantly affecting cardiac muscle
Niemann-Pick disease type C1	an autosomal recessive lipid storage disorder caused by mutation in the NPC1 gene and characterized by progressive neurodegeneration
Wolman disease	early-onset fulminant disorder of infancy with massive infiltration of the liver, spleen, and other organs by macrophages filled with cholesteryl esters and triglyceridess. It is caused by homozygous or compound heterozygous mutation in the LIPA gene
Gaucher disease	an autosomal recessive lysosomal storage disorder due to deficient activity of beta-glucocerebrosidase
Multiple Sulfatase Deficiency	an autosomal recessive inborn error of metabolism caused by homozygous or compound heterozygous mutation in the sulfatase-modifying factor-1 gene (SUMF1)
Mucopolysaccharidosis	a group of metabolic disorders caused by the absence or malfunctioning of lysosomal enzymes needed to break down molecules
Autophagosome	intracytoplasmic vacuole containing elements of a cell's own cytoplasm; it fuses with a lysosome and the contents are subjected to enzymatic digestion
lysosome-related organelles	cell type-specific compartments, which include melanosomes, lytic granules, MHC class II compartments, platelet-dense granules, basophil granules, azurophil granules, and Drosophila pigment granules
Fronto-Temporal Dementia	refers to a clinical manifestation of the pathologic finding of frontotemporal lobar degeneration
Charcot-Marie-Tooth type 2B	autosomal dominant peripheral sensory neuropathy due to mutations in the small GTPase late endosomal protein RAB7
Neuronal Ceroid Lipofuscinosis	neuronal ceroid lipofuscinoses (NCL; CLN) are a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by the intracellular accumulation of autofluorescent lipopigment storage material
Pompe disease	Glycogen storage disease II, an autosomal recessive disorder, is the prototypic lysosomal storage disease

caused by mutation in the gene encoding acid alpha-1,4-glucosidase

Box 1

The structure of the lysosome

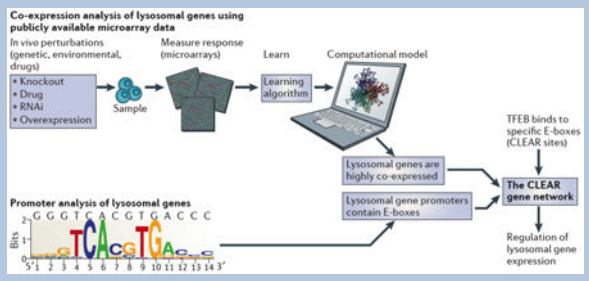
Lysosomes have a limiting membrane, which is composed of a single lipid bilayer and integral and peripheral proteins, and an acidic lumen that contains soluble hydrolytic enzymes and activators. [47, 49, 194, 195]. A glycocalyx lines the internal lysosomal perimeter, protecting the membrane from the acidic environment of the lumen. Soluble enzymes are directly involved in the degradation of metabolites, while the lysosomal membrane segregates this catalytic potential and also actively participates in the maintenance of membrane integrity, the establishment of lumen acidic pH, metabolite and ion membrane 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 LAMP1, which is the most abundant lysosomal membrane protein accounting for 50% of this membrane's total protein. 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 (vATPase), 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 (TRPML1-MCOLN1) is a non-selective cation channel[199] that is involved in calcium signalling during lysosomal fusion with other membranes, such as the plasma membrane[85, 87] and autophagosomes[200]. A deficiency in mucolipin-1 causes mucolipidosis IV, a lysosomal storage disease[201, 202]. CIC-7, a chloride 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 (CMA) by binding cytosolic protein substrates to the lysosomal membrane so that they can be internalized into lysosomes for degradation[5, 204]. Mutations of LAMP2A cause Danon disease, which is associated with the accumulation of autophagic vacuoles in muscle cells[205]. NPC1 is a lysosomal membrane protein involved in the export of cholesterol from the endo-lysosomal compartment and it is mutated in Niemann-Pick disease type C1[206]. The recently identified lysosomal amino acid transporter 1 (LAAT-1) is involved in the transport of lysine and arginine across the lysosomal membrane and into the lysosome and it plays a crucial role in cellular amino acid homeostasis[207]. Enzymes on the lysosomal membrane include HEPARAN-alpha glucosaminide N-acetyltransferase (HGSNAT). This enzyme participates in the stepwise degradation of heparan sulfate[208-210] and mutation of this protein causes mucopolysaccharidosis type IIIC.



Box 2

The identification of the CLEAR gene network

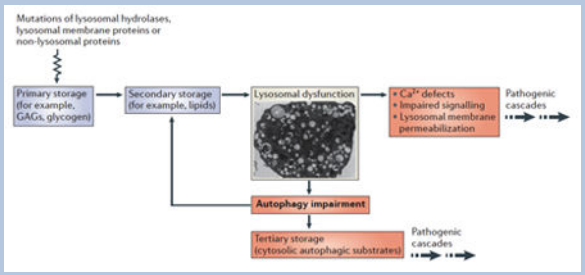
Gene networks control several aspects of cellular function and metabolism, such as the coordination of the cellular response to environmental conditions. In specialized organelles, this coordination is facilitated by compartmentalization. A systems biology approach was used to test the hypothesis that 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 a variety of different tissues and cell types and 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 the transcription factor EB (TFEB), which belongs to the MITF 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 gene of the CLEAR network[12].



Box 3

Mechanisms of lysosomal storage diseases (LSDs)

LSDs are a group of individually rare, recessively inherited, inborn errors of metabolism with an overall incidence of 1 in 5000, 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 a variety of undegraded material in the lysosomes of most cells and tissues. Approximately 60 different types of LSDs have been recognized. Historically, LSDs have been classified based on the type of material that accumulates in the lysosomes, such as mucopolysaccharidoses, sphingolipidoses, glycoproteinoses, glycogenosis, and lipofuscinoses. LSDs often show a multisystemic phenotype associated with severe neurodegeneration, mental decline, cognitive problems, and behavioral 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 (see the figure). Mutations in genes that are important for lysosomal function result in the accumulation of specific undegraded substrates in the lysosome (primary storage). This leads to a secondary 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, signaling abnormalities, and lysosomal membrane permeabilization. In addition, lysosomal dysfunction is associated with autophagy impairment, due to a defect in the fusion between lysosomes and autophagosomes, causing the “tertiary storage” of autophagy substrates (such as aggregate prone proteins and dysfunctional mitochondria), both of which contribute to neurodegeneration.



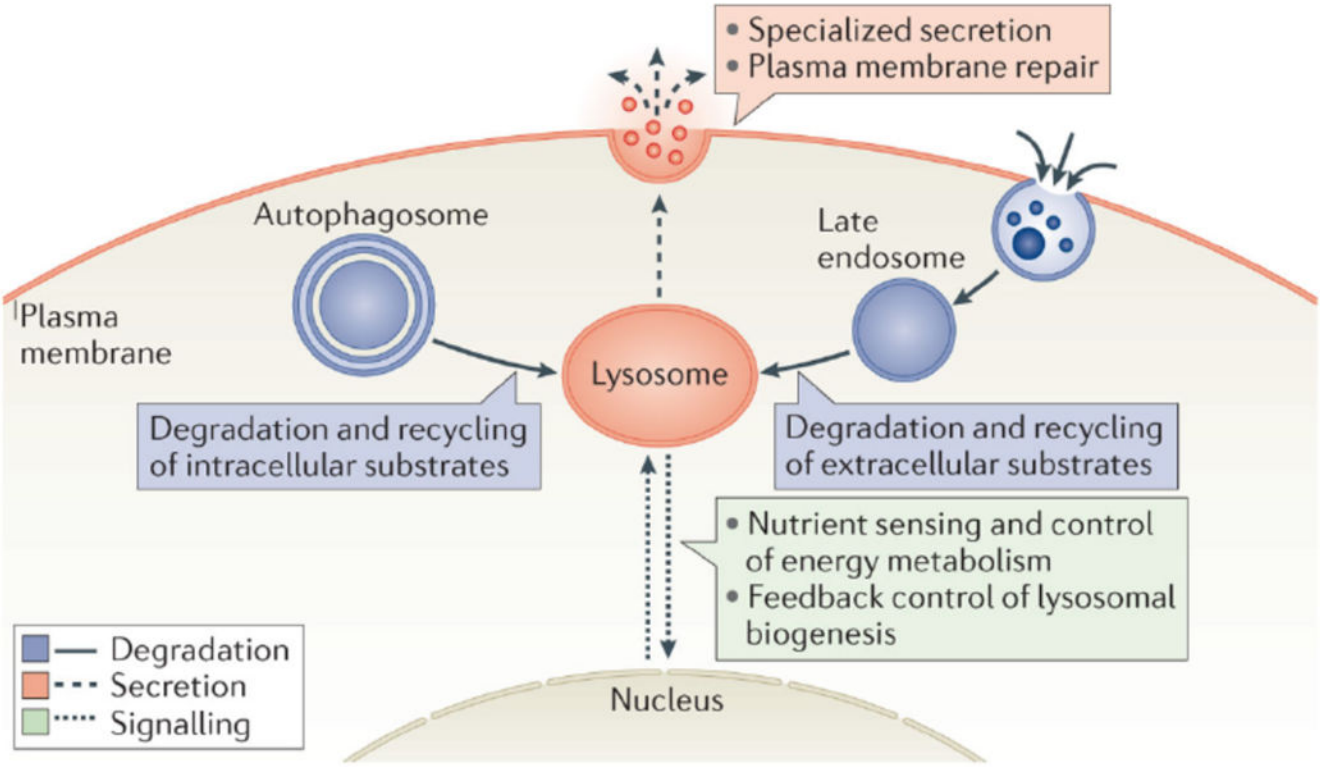


Figure 1. Main functions of the lysosome and their relationship with key cellular processes

Lysosomes are involved in the degradation and recycling of extracellular material, via endocytosis, and intracellular material, via autophagy. In these processes lysosomes fuse with late endosomes and with autophagosomes, 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 membrane. 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 signaling organelles that can sense nutrient availability and activate a lysosome-to-nucleus signaling pathway that mediates the starvation response and regulates energy metabolism.

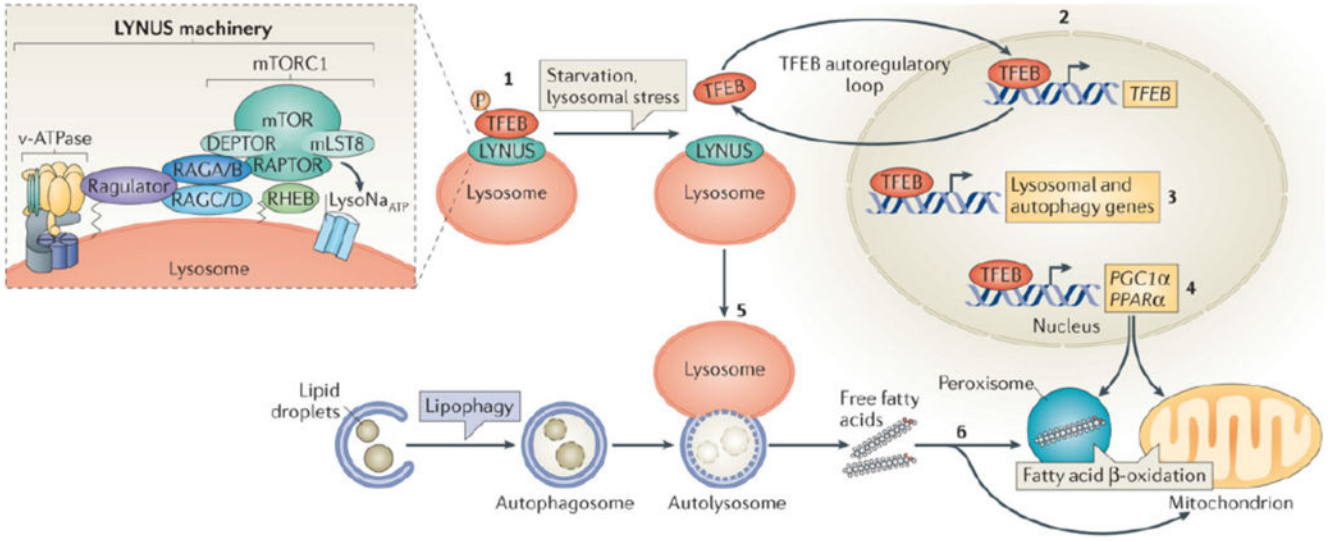


Figure 2. Model of TFEB regulation and function during starvation

This model illustrates how transcription factor EB (TFEB) is induced by starvation and mediates the starvation response by regulating lipid catabolism. In the presence of adequate nutrition TFEB interacts with the lysosome nutrient sensing (LYNUS) machinery, which senses lysosomal nutrient levels via the vATPase complex, and is phosphorylated by 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 it 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). and of PGC1α–PPARα target genes (4). In this way TFEB controls the starvation response by activating both macrolipophagy (5) and fatty-acid oxidation (6). The **insert** shows the main components of the LYNUS machinery. The mTORC1 complex, which includes regulatory proteins associated with mTOR, such as RAPTOR, LST8, and DEPTOR[211], physically interacts with the RAG GTPases on the lysosomal surface and it is activated by them[212]. A complex known as the Ragulator mediates the activation and docking of RAGS to the lysosomal membrane[97, 213] and the small GTPase RHEB is also involved in the growth factor-mediated activation of mTORC1[214, 215]. The vATPase complex is involved in amino acid sensing and it mediates amino acid-sensitive interactions between Rags and Ragulator, which is the initial step in lysosomal signaling[104]. The ATP-sensitive Na^+ channel $lysoNa_{ATP}$, which is comprised of the subunits TPC1 and TPC2, is located on the lysosomal membrane and it has recently been shown to interact with mTORC1 and participates in nutrient sensing[106] The nature of interaction between $lysoNa_{ATP}$ and mTORC1 is unknown but seems to be independent form other components of the LYNUS machinery and the transcription factor EB (TFEB) and its interacting proteins (see text).

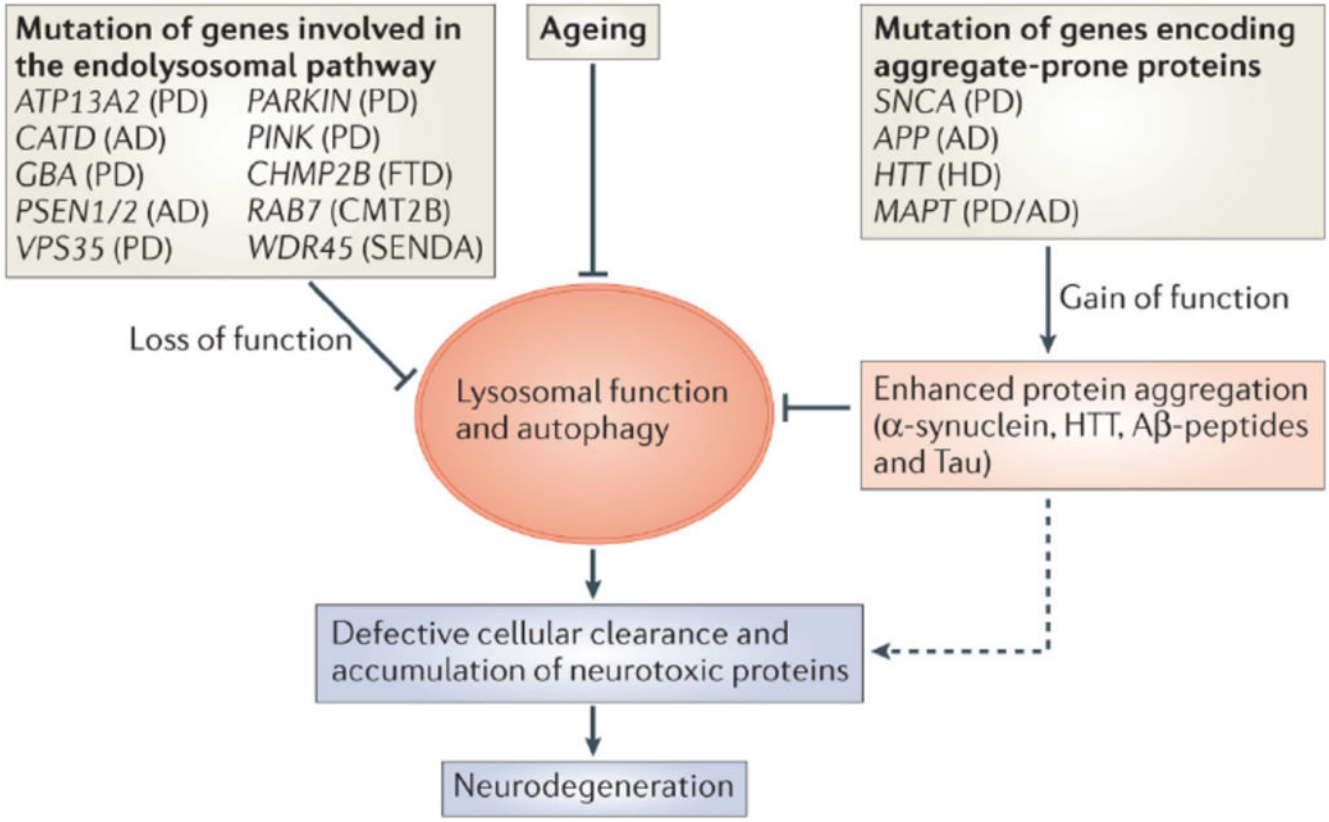


Figure 3. Defective cellular clearance in neurodegenerative diseases

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, CATD, GBA1, PSEN1/2, VPS35, PINK, PARK, CHMP2B, RAB7, and WDR45) can affect cellular degradation and recycling processes. Second, gain-of-function mutations of aggregate-prone proteins (for example, SNCA, APP, HTT and MAPT) may lead to enhanced protein aggregation and engulfment 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. (PD = Parkinson's disease; AD = Alzheimer's diseases; FTD = Fronto-Temporal Dementia; CMT2b = Charcot-Marie-Tooth type 2B; SENDA = Static Encephalopathy of Childhood with Neurodegeneration in Adulthood).

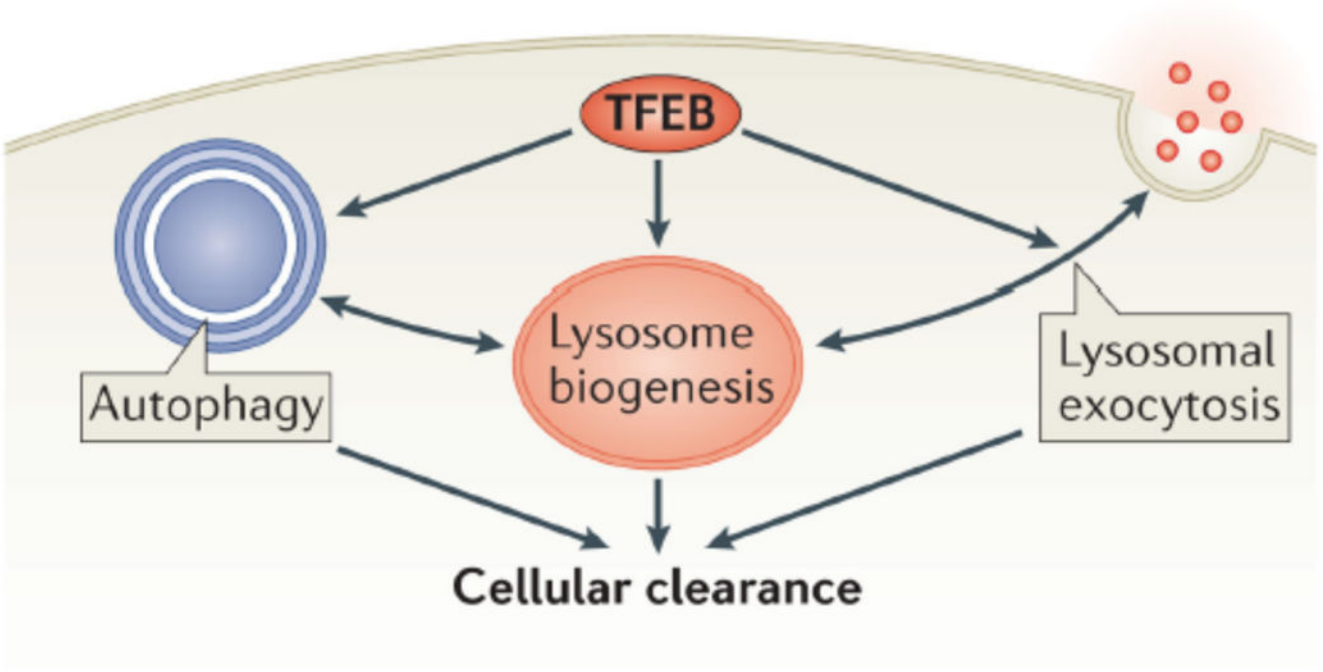


Figure 4. TFEB regulates cellular clearance

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 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. TFEB