

## Review

## Molecular Mechanisms of Lysosome and Nucleus Communication

Qian Zhao,<sup>1,3</sup> Shihong Max Gao,<sup>1,2,3</sup> and Meng C. Wang<sup>1,2,3,4,\*</sup>

**Lysosomes transcend the role of degradation stations, acting as key nodes for interorganelle crosstalk and signal transduction. Lysosomes communicate with the nucleus through physical proximity and functional interaction. In response to external and internal stimuli, lysosomes actively adjust their distribution between peripheral and perinuclear regions and modulate lysosome–nucleus signaling pathways; in turn, the nucleus fine-tunes lysosomal biogenesis and functions through transcriptional controls. Changes in coordination between these two essential organelles are associated with metabolic disorders, neurodegenerative diseases, and aging. In this review, we address recent advances in lysosome–nucleus communication by multi-tiered regulatory mechanisms and discuss how these regulations couple metabolic inputs with organellar motility, cellular signaling, and transcriptional network.**

**Lysosomes as Degradation and Signaling Hubs**

Lysosomes were first discovered by the Nobel laureate Christian de Duve in 1955 as ‘scientific serendipity’ [1], were later found in nearly all eukaryotic cells (vacuoles in plants and yeasts; lysosomes in animals), and have the reputation of being ‘waste-to-energy’ incinerators inside the cell. Enclosed in a single phospholipid bilayer membrane, lysosomes maintain an acidic interior (pH 4.5–5.5) by pumping cytosolic protons into the internal lumen via vacuolar H<sup>+</sup>-ATPase, which is aided by other lysosomal ion channels [2]. The acidic lumen of lysosomes accommodates ~60 acidic hydrolases and provides them with an optimal environment in which to break down various biological macromolecules [3,4]. These biological macromolecules are delivered into lysosomes from the extracellular or intracellular space through **endocytosis** (see [Glossary](#)) or autophagy ([Box 1](#)), respectively [5–7], and their degradation products, such as amino acids, fatty acids (FAs), monosaccharides, and nucleosides, are exported to the cytosol for reuse via lysosomal transporters and/or vesicle trafficking [8–10]. In several specialized cell types, lysosomes are a critical part of the secretory pathway known as lysosomal **exocytosis**, which is essential for plasma membrane repair and cholesterol homeostasis [11–14].

In addition to their well-appreciated housekeeping function as cellular degradative compartments, lysosomes are now emerging as crucial signaling hubs that relay external and internal stimuli, such as growth factors, nutrient availability, and amino acid, glucose, and lipid metabolism, to signaling complexes, such as mammalian/mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK), and undergo adaptation to meet cellular needs [15–17]. The adaptation of lysosomes occurs at multiple layers, and the communication between the lysosome and the nucleus has a crucial role in this process. Here, we overview three regulatory layers of lysosome–nucleus communication, including the peripheral–perinuclear positioning of lysosomes, nuclear translocation of signaling factors from lysosomes, and transcriptional control of **lysosomal adaptation** by nuclear factors. We highlight the molecular players that are involved in these regulations, how these regulations couple with metabolic and environmental changes,

## Highlights

Lysosomal–nuclear communication coordinates lysosomal positioning, metabolism, and signaling with nuclear transcription to support fundamental cellular activities.

Lysosomal positioning between peripheral and perinuclear regions is subject to cooperative regulation in response to metabolic stimuli and contributes to lysosomal functions and signaling.

Retrograde transport of chaperone proteins and transcription factors from the lysosome to the nucleus is actively regulated by metabolic signaling.

Nuclear transcriptional networks comprise a variety of different transcription factors that act independently or coordinately to fine tune lysosomal-autophagic gene expression.

<sup>1</sup>Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA

<sup>2</sup>Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, USA

<sup>3</sup>Huffington Center on Aging, Baylor College of Medicine, Houston, TX 77030, USA

<sup>4</sup>Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA

\*Correspondence: [wmeng@bcm.edu](mailto:wmeng@bcm.edu) (M.C. Wang).



### Box 1. Lysosomes in Autophagy

In response to nutrient deprivation, autophagy is a highly regulated catabolic process that captures unwanted intracellular components, including macromolecules and even entire organelles, and delivers them to lysosomes for bulk degradation and recycling. Three major types of autophagy have been identified in eukaryotes: (i) chaperone-mediated autophagy (CMA); (ii) microautophagy; and (iii) macroautophagy. CMA and microautophagy both take place directly on lysosomes. In CMA, protein substrates with KFERQ-like motifs are recognized by HSC70 and co-chaperones and internalized into the lysosome with the aid of lysosome-associated membrane protein type 2A (LAMP2A) [108–110]. In microautophagy, cytosolic materials are directly captured into the lysosome via the invagination of lysosomal membrane [111].

By contrast, macroautophagy, a major type of autophagy, requires extensive membrane remodeling and the formation of an autophagosome, a specialized double membrane-bound vesicle that engulfs cytoplasmic contents and delivers these cargos to the lysosome through direct fusion (autolysosome) [112,113]. Upon fusion, the cargos are degraded inside the acidic lumen of the autolysosome by various hydrolases for recycling. All three forms of autophagy depend on the proper function of lysosomes.

and how these discoveries have broadened our view of lysosomal contributions to health, disease, and aging (Box 2).

### Peripheral–Perinuclear Positioning of Lysosomes

Lysosomes are broadly distributed in the cytoplasm and can change positioning in response to different metabolic and signaling inputs [18]. In particular, movement from the cell periphery toward the perinuclear region (retrograde) and from the perinuclear region toward the cell periphery (anterograde) contributes to lysosome-related cellular processes, including cell migration, autophagy, and immune responses, as well as to the regulation of lysosomal signaling pathways [19]. The intracellular movement of lysosomes is governed by two families of microtubule-based motors: kinesins and dynein. Generally, in an unpolarized cell, kinesins move toward the microtubule plus end (toward the cell periphery), while dynein moves toward the minus end (toward the nucleus or microtubule-organizing center) [20,21]. Perinuclear movement of

### Box 2. Lysosome Dysfunction and Disease

Dysfunction of lysosomes has been associated with multiple human diseases. Mutations in lysosomal hydrolase and/or transporter genes result in aberrant accumulation of a variety of metabolites within the lysosomal lumen, leading to metabolic diseases collectively called lysosomal storage diseases (LSDs) [107,114,115]. Dysfunction of lysosomal-autophagic pathways is detected in neurodegenerative diseases, such as Parkinson's, Alzheimer's, and Huntington's diseases, and amyotrophic lateral sclerosis (ALS), which share a common feature in the form of neuronal disposition of protein aggregates [116–119]. Defects in lysosome trafficking machinery are also involved in LSDs and neurodegenerative diseases, as reviewed in [120]. Overexpression of transcription factor (TF)EB/TFE3 stimulates lysosomal exocytosis and promotes the clearance of undigested materials in several cell models of LSDs [64,121,122]. Overexpression of TFEB or pharmacological stimulation of its activity also offers protection against the pathology of Parkinson's, Alzheimer's, and Huntington's diseases in mouse models [62,123–126]. In invertebrate model organisms, the nuclear retention of TFEB caused by the mutation of a nuclear export protein **exportin 1 (XPO1)** enhances autophagy and protects ALS-afflicted fruit flies from developing neurodegeneration [127].

Despite the protection against LSDs and neurodegenerative diseases, the enhanced lysosomal-autophagic process caused by TFEB/TFE3 overexpression can drive tumor progression by recycling internal cellular constituents in cancer cells and compensating for the shortage of external nutrients due to insufficient vascularization [128,129]. For instance, pancreatic ductal adenocarcinoma (PDA) exhibits constitutive activation of microphthalmia-associated transcription factor (MITF) TFs to maintain the amino acid pool derived from autophagy [130] and, consequently, the pathogenesis of PDA requires a high level of autophagy [131]. TFE-fusion renal cell carcinomas (TFE-fusion RCCs) result from chromosomal translocation and MITF gene fusion that lead to the overexpression of the TFEB and TFE3-encoding genes [132,133]. By contrast, Forkhead box O1 (FOXO1) was shown to activate the autophagic process, which leads to cell death and protection against human colon cancer [84,133].

Moreover, lysosomal-autophagic activity decreases with age. FOXO and TFEB can coregulate lysosome biogenesis and autophagy genes [85,86]; their increased activities contribute to lifespan extension in diverse organisms [76,134,135] and protection against age-associated diseases, such as osteoarthritis [85].

### Glossary

**14-3-3:** a family of structurally similar intracellular phosphor-serine/threonine binding proteins found in all eukaryotes.

**ADP ribosylation factor-like 8 (Ar18):** a small G protein and regulator of lysosomal anterograde trafficking.

**AKT:** also known as protein kinase B; a serine/threonine kinase and main regulator of metabolism.

**BLOC-1-related complex (BORC):** a multisubunit complex that regulates lysosome positioning.

**cAMP response element-binding protein (CREB):** a transcription factor.

**Endocytosis:** process by which cells internalize materials surrounding the cell membrane through membrane invagination and formation of vesicles.

**Epidermal growth factor receptor substrate 15 (EPS15):** an endocytic adaptor protein interacting with RNF26/SQSTM1.

**Exocytosis:** an energy-dependent secretory process that transports cellular materials confined within vesicles out of the cell membrane into the extracellular space.

**Exportin 1 (XPO1):** a eukaryotic nuclear export protein.

**Extracellular signal regulated protein kinase (ERK):** member of the mitogen-activated protein kinase family.

**FYVE and coiled-coil domain-containing protein 1 (FYCO1):** a regulator of lysosomal retrograde transportation.

**Glycogen synthase kinase 3 (GSK3):** a serine/threonine protein kinase.

**Lipolysis:** biochemical pathway through which ester bonds in neutral lipids and triglycerides are hydrolyzed, resulting in the generation of fatty acids and glycerol.

**Lysosome adaptation:** lysosomes actively adjust their biosynthesis and activities in response to intracellular and environmental inputs, which is transcriptionally coordinated by multiple factors.

**Microphthalmia-associated transcription factor (MITF):** member of a family of basic helix-loop-helix leucine zipper transcription factors.

**Mitochondria fission 1 protein (FIS1):** is a regulator of mitochondrial fragmentation.

**Oxysterol-binding protein (OSBP):** a receptor for a variety of oxysterols.

**Oxysterol-binding protein-related protein 1 (ORP1L):** member of the human OSBP family.

lysosomes has been observed in a variety of lysosomal storage diseases (LSDs) (Table 1) and can be triggered upon nutrient deprivation, which is the key input modulating lysosomal signaling. This movement is governed by multiple cooperative mechanisms, including the enhancement of coupling with dynein and its activator dynactin, the promotion of tethering between lysosomes and the perinuclear endoplasmic reticulum (ER)/Golgi complex, and the inhibition of anterograde transport involving coupling lysosomes to kinesins (Figure 1).

#### Perinuclear Retrograde Movement Associated with Dynein–Dynactin

Three independent mechanisms have been reported to couple lysosomes with the dynein–dynactin retrograde motor. First, the lysosome-associated small GTPase Rab7 can interact with a homodimeric effector protein Rab7-interacting lysosomal protein (RILP), which subsequently recruits the p150<sup>Glued</sup> subunit of the dynein–dynactin complex and drives lysosomal distribution toward the perinuclear region [22] (Figure 1, pathway A2). RILP is also in association with **oxysterol-binding protein-related protein 1** (ORP1L), which can adopt different conformations in response to the cholesterol level of the lysosome and, in turn, modulates the formation of lysosome–ER contact sites. In normal conditions, the ER protein VAP interacts with the Rab7-RILP complex at those contact sites and competes with p150<sup>Glued</sup> and dynein–dynactin [23]. Upon cholesterol accumulation induced by chemical or genetic intervention, ORP1L adopts a conformation that prevents the formation of the lysosome–ER contact and, consequently, the interaction between VAP and the Rab7-RILP complex, leading to the perinuclear movement of lysosomes [23].

Second, the lysosomal calcium channel **transient receptor potential, mucolipin subfamily, member 1** (TRPML1) can bind with the calcium sensor ALG2 [24], which physically associates with dynein–dynactin and mediates the retrograde transport and perinuclear accumulation of lysosomes [25] (Figure 1, pathway A3). TRPML1 is the predominant calcium channel that controls calcium release in the lysosome [26] and can be activated by lysosome-specific phosphatidylinositol 3,5-bisphosphate [PI(3,5)P<sub>2</sub>] [27], the biosynthesis of which is regulated by both nutrient and growth factors [28]. In response to starvation, autophagy induction, or cytosolic alkalization, lysosomes undergo perinuclear redistribution, which is blocked by acute TRPML1 inhibition or PI(3,5)P<sub>2</sub> depletion [25]. Conversely, the activation of TRPML1 sufficiently induces perinuclear accumulation of lysosomes in a PI(3,5)P<sub>2</sub> and ALG-2 dependent manner [25]. Notably, although mutations of human *TRPML1* are linked with mucopolipidosis type IV (ML-IV) LSD [26], the TRPML1-ALG-2 mechanism does not contribute to the perinuclear accumulation of lysosomes in ML-IV; neither does it contribute to the perinuclear accumulation of lysosomes in Niemann–Pick disease type C1, another LSD characterized by cholesterol accumulation [25].

#### Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α)

is a transcriptional co-activator for several nuclear receptors.

**Ring finger protein 26 (RNF26):** an ER-associated E3 ubiquitin ligase.

**SifA-kinesin interacting protein (SKIP):** also known as PLEKHM2; an adaptor protein between kinesin motor protein and ARL-8.

**Tax1-binding protein 1 (TAX1BP1):** an autophagy receptor and vesicle adaptor protein interacting with RNF26/SQSTM1.

**TBC1 domain family member 15 (TBCF1D15):** a GTPase activation protein for Rab7.

**Toll-interacting protein (TOLLIP):** negative regulator of Toll-like receptor-mediated signaling as well as a vesicle adaptor protein interacting with RNF26/SQSTM1.

**Transient receptor potential, mucolipin subfamily, member 1 (TRPML1):** a cation-permeable channel that is localized on both late endosomes and lysosomes.

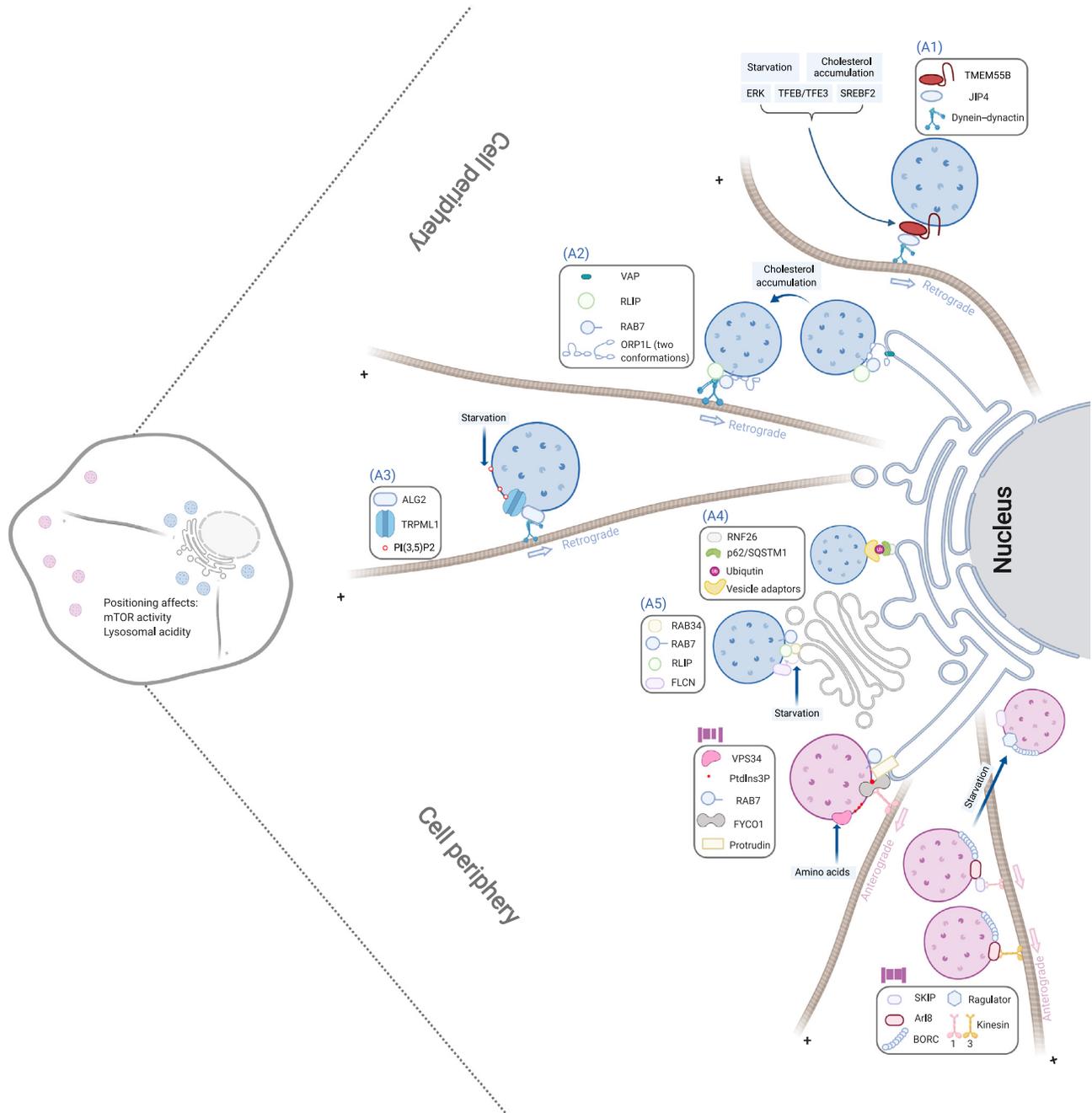
**Ubiquitin carboxyl-terminal hydrolase 15 (USP15):** a deubiquitinating enzyme.

**Vacuolar protein sorting 34 (VPS34):** a class 3 phosphatidylinositol (PtdIns) 3-kinase and an endosomal trafficking regulator.

Table 1. Lysosome Positioning Changes in Human Disease Models

Disease	Lysosome positioning <sup>a</sup>	Model system	Refs
Mucopolipidosis type IV	Perinuclear	Primary mouse fibroblasts	[25]
Niemann–Pick type C	Perinuclear	Primary mouse cell isolations	[136]
		Patient-derived fibroblasts	[137]
Juvenile CLN3	Perinuclear	HeLa cells	[138]
Huntington's	Perinuclear	Mouse striatal-derived cell line	[139]
	Axonal/Peripheral <sup>b</sup>	Mouse primary dorsal root ganglion neurons	[140]
Alzheimer's	Axonal/Peripheral <sup>c</sup>	Mouse hippocampus	[141,142]

<sup>a</sup>A broad definition of lysosomes, including lysosomes, autophagosomes<sup>b</sup> and amphisomes<sup>c</sup> (fusion between autophagosomes and endosomes), is applied.



## Trends in Biochemical Sciences

**Figure 1. Molecular Mechanism of Lysosome Positioning.** The perinuclear clustering of lysosomes (marked in blue) is driven by the following machineries: (A1) TMEM55B binding with the dynein adaptor JIP4 to recruit the dynein–dynactin complex; (A2) oxysterol-binding protein-related protein 1 (ORP1L) adopting a conformation to facilitate the detachment of lysosomes from the endoplasmic reticulum (ER) and the recruitment of dynein–dynactin by the Rab7–Rab7-interacting lysosomal protein (RILP) complex; (A3) lysosomal lipid phosphatidylinositol 3,5-bisphosphate [PI(3,5)P<sub>2</sub>] activating the transient receptor potential, mucolipin subfamily, member 1 (TRPML1) calcium channel to allow the recruitment of dynein–dynactin by the calcium sensor ALG2; (A4) ER-located ring finger protein 26 (RNF26) ubiquitinating p62/SQSTM1 and recruiting various vesicle adaptors through their ubiquitin-binding domains; and (A5) lysosome-associated folliculin (FLCN) promoting the interaction between lysosomal Rab7 and RILP and Golgi-localized Rab34. By contrast, periphery positioning of lysosomes (marked in pink) is mediated by (B1) vacuolar protein sorting 34 (VPS34)-induced phosphatidylinositol 3-phosphate (PtdIns3P) to promote the transfer of kinesin-1 from ER protrudin to lysosomal FYVE

(Figure legend continued at the bottom of the next page.)

Third, lysosomal transmembrane protein TMEM55B can directly bind with the dynein scaffold protein JIP4 and recruit dynein–dynactin to lysosomes through JIP4, inducing lysosomal retrograde transport and perinuclear positioning [29] (Figure 1, pathway A1). *TMEM55B* overexpression causes the clustering of lysosomes around the nucleus, while its knockdown increases the concentration of lysosomes in the cell periphery [29]. Interestingly, starvation upregulates the expression of *TMEM55B* through the transcription factor EB (TF)EB/TFE3, the master regulator of lysosomal biogenesis [29,30] (see ‘Transcriptional Control of Lysosomal Adaptation’). The transcription of *TMEM55B* also responds to cholesterol levels, mediated by SREBF2 and TFEB/TFE3 [29]. As a result, the TMEM55B–JIP4 mechanism contributes to the perinuclear redistribution of lysosomes caused by chemical and genetic induction of lysosomal cholesterol accumulation [29]. In addition to the transcriptional control, the TMEM55B–JIP4 mechanism is also subject to post-transcriptional regulation by **extracellular signal regulated protein kinase** (ERK) [29,30].

#### Perinuclear Retrograde Movement via ER/Golgi Interaction

Additionally, lysosomal positioning close to the perinuclear region is mediated by the interaction of lysosomes with ER and Golgi. Rab34, a small GTPase primarily localized to the perinuclear Golgi complex, can interact with RILP [31] and Rab7 to promote lysosome–Golgi tethering and perinuclear retention of lysosomes [32] (Figure 1, pathway A5). Rab34 is required for starvation-induced perinuclear accumulation of lysosomes and is sufficient to promote lysosome movement toward the nucleus upon overexpression [32]. Lysosome-associated folliculin (FLCN) facilitates this regulatory mechanism by interacting with RILP and promoting the loading of active Rab34 onto RILP upon starvation [32].

At the same time, an ER-located ubiquitin ligase **ring finger protein 26** (RNF26) can recruit and ubiquitinate p62/SQSTM1 (the ubiquitin-binding protein involved in autophagosome cargo recruitment), which further attracts ubiquitin-binding domains of various vesicle adaptors, including **epidermal growth factor receptor substrate 15** (EPS15), **Tax1-binding protein 1** (TAX1BP1), and **Toll-interacting protein** (TOLLIP), and restricts their cognate vesicles in the perinuclear region [33] (Figure 1, pathway A4). This anchoring process is terminated upon deubiquitination of p62/SQSTM1 by the RNF26-associated deubiquitinating enzyme **ubiquitin carboxyl-terminal hydrolase 15** (USP15), which facilitates vesicle release toward the periphery [33]. Depletion of RNF26, or knocking down of p62/SQSTM1, EPS15, TAX1BP1, or TOLLIP, leads to the redistribution of lysosomes toward the peripheral region, while depletion of USP15 decreases the number of periphery-located lysosomes and overall endosome mobility [33]. It is currently unclear whether the activity of RNF-16 and/or UPS15 is modulated by the nutrient status of the cell.

#### Peripheral Anterograde Transport with Kinesins

Starvation also inhibits anterograde transport of lysosomes toward the periphery. Anterograde transport can be mediated by a lysosomal eight-unit complex **BLOC-1-related complex** (BORC), which recruits a small GTPase, **ADP ribosylation factor-like 8** (Arl8), to the lysosomal membrane [20] (Figure 1, pathway B2). Arl8 in turn interacts with different kinesins to drive the peripheral movement of lysosomes [34]. Arl8 requires an effector protein **SifA-kinesin interacting protein** (SKIP) to establish the connection with kinesin-1 [35] but directly binds to kinesin-3 [20]. Knocking down of Arl8, SKIP, BORC, kinesin-1, or kinesin-3 causes perinuclear redistribution of

---

and coiled-coil domain-containing protein 1 (FYCO1) and (B2) BLOC-1-related complex (BORC) recruitment of ADP ribosylation factor-like 8 (Arl8) to interact with different kinesins, which is blocked by its interaction with Ragulator. Each of these pathways can be regulated by different stimuli, as indicated by the light-blue boxes, and lysosomal positioning regulates lysosomal acidity and mammalian target of rapamycin (mTOR) activation. These pathways are shown as independent for presentation clarity, but multiple machineries can be present in a single lysosome.

lysosomes without starvation [20,34,35]. During starvation, BORC interaction with the Ragulator complex is enhanced, which prevents BORC-Arl8b binding and consequently leads to lysosomal accumulation around the nucleus [21]. However, overexpression of Arl8 or kinesin-1 can block this starvation-induced perinuclear accumulation [36].

At the same time, anterograde movement can be regulated by phosphatidylinositol 3-phosphate (PtdIns3P), which promotes lysosome–ER contact and the translocation of lysosomes to the cell periphery [37] (Figure 1, pathway B1). PtdIns3P on the lysosome recruits a PtdIns3P-binding protein, **FYVE and coiled-coil domain-containing protein 1** (FYCO1), to the lysosome membrane and another PtdIns3P-binding protein, Protrudin, to the ER membrane, which facilitates the transfer of kinesin-1 from protrudin to FYCO1 to initiate anterograde transportation [37,38]. The production of PtdIns3P on the lysosome is catalyzed by the lipid kinase **vacuolar protein sorting 34** (VPS34), which is stimulated by an increased level of amino acids [39]. Thus, upon starvation, this PtdIns3P-dependent anterograde transportation mechanism is comprised due to inactive VPS34.

Collectively, the emerging research on lysosome positioning starts to reveal the tip of the iceberg for this delicately balanced system with high dynamics. Positioning changes have been reported to affect lysosomal acidity and autophagic flux [36,40,41] as well as signal transduction (see 'Lysosomal Positioning and Signaling Activation'). Further understanding of its regulatory mechanisms will advance our current understanding of the roles of lysosomes in cellular signaling, organismal development, pathogenesis, and aging.

### Lysosome–Nucleus Interactions in Signaling Transduction

Importantly, the interactions between lysosomes and the nucleus do not just occur at the morphological level. Dependent on their abilities to cluster at the perinuclear region, produce various metabolites, and anchor signaling factors, lysosomes can communicate with the nucleus to regulate signal transduction and transcription responses. Three examples of such regulations are highlighted here.

#### Lysosomal Positioning and Signaling Activation

As a master regulator of cellular growth and metabolism, mTOR complex 1 (mTORC1) has crucial roles in cancer, neurodegeneration, metabolic disorders, and aging. Recent studies revealed mTORC1 activation at the lysosomal surface, which is regulated by Ragulator, GATOR1, and FLCN in complex with FNIP (reviewed in [42–44]), and also the interesting link between mTORC1 activation and lysosomal positioning [36]. On the one hand, mTORC1 influences lysosomal positioning in response to nutrient and growth factors (see earlier and Figure 1, pathways B2 and A5). More specifically, Ragulator physically interacts with BORC and, in turn, comprises its ability to recruit kinesins, which is enhanced upon starvation or weakened by growth factors to drive the perinuclear or peripheral movement of lysosomes, respectively [21,45]. Meanwhile, direct binding of FLCN with RILP, in response to starvation, promotes the loading of active Rab34, the tethering of lysosomes to the perinuclear Golgi complex, and the retaining of lysosomes around the nucleus [32]. On the other hand, lysosomal positioning can in turn influence mTORC1 activation. Overexpression of proteins mediating the anterograde transport of lysosomes, such as BORC, Arl8, Protrudin, and FYCO1, enhances mTORC1 activity [36,38]. Conversely, genetic knockdown or knockout of those anterograde transport mediators attenuate mTORC1 activation in response to nutrient and growth factors [36,38,46].

However, there are also studies that associate peripheral positioning of lysosomes with a reduction in mTORC1 activity [47,48]. For example, cytosolic acidification resulting from hypoxia leads

to a peripheral distribution of lysosomes and mTORC1 inhibition [48]. This controversy might be related to specific mechanisms activating mTORC1 under different conditions. In response to growth factors, perinuclear clustering of lysosomes attenuates the activation of mTORC2 and **AKT**, which can augment mTORC1 activation [46]; while, in response to cytosolic acidification, peripheral redistribution of lysosomes drives lysosome-bound mTOR away from the perinuclear pool of RHEB that activates it [48].

#### Nuclear Translocation of Lysosomal FABPs

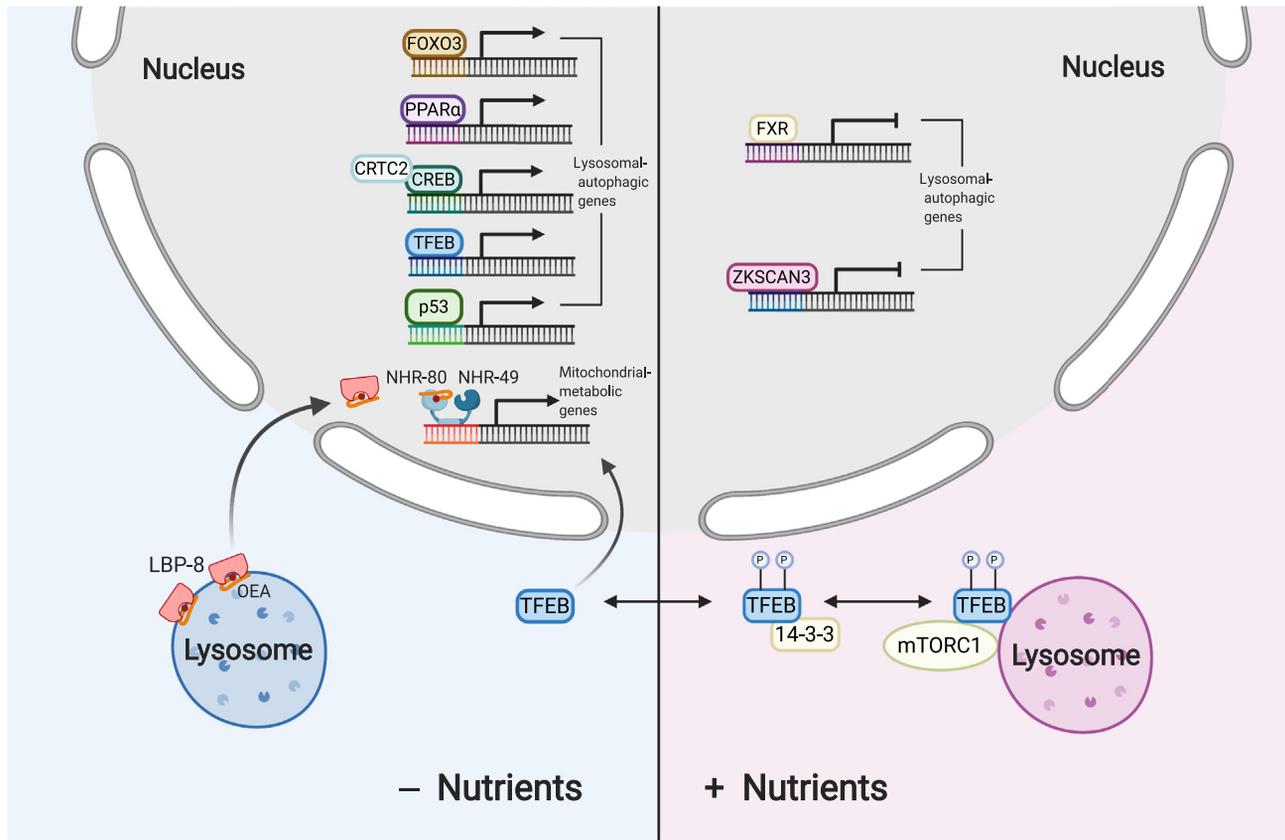
As a metabolic active site, lysosomes are responsible for the digestion and degradation of macromolecules and the production of various metabolites. A crucial group of bioactive metabolites comprise FAs and FA derivatives that are sources of energy fuel, membrane building, and signal transduction [49]. Within the lysosomal lumen, acidic lipases and phospholipases can de-esterify neutral lipids and phospholipids, respectively, to generate FAs and FA derivatives, which may be exported via vesicular routes and/or lipid-binding proteins and transporters [50].

In the cytoplasm, hydrophobic FAs and FA derivatives rely on FA-binding proteins (FABPs) to facilitate their transportation between organelles [51]. Therefore, this family of proteins acts as lipid chaperones to mediate the metabolic and signaling effects of FAs and FA derivatives [51]. In particular, specific FABPs can selectively carry FAs to the nucleus and activate a family of nuclear hormone receptors known as peroxisome proliferator-activated receptors (PPARs) [52]. FABP proteins are conserved from *Caenorhabditis elegans* to humans, with nine homologs in each organism. One of the *C. elegans* FABPs, LBP-8, is reported to be translocated from the lysosome to the nucleus upon lysosomal **lipolysis** induced by overexpression of a lysosomal acid lipase LIPL-4 [53]. The LBP-8 protein is able to bind a variety of FAs and FA derivatives [53,54], and carries a structurally conserved nuclear localization signal [54]. Overexpression of LIPL-4 also leads to an increase in oleoylethanolamide (OEA), a FA derivative that directly binds to LBP-8 and requires its nuclear translocation to activate the nuclear hormone receptor complex NHR-80 and NHR-49 (Figure 2) [53,54]. This complex in turn upregulates *lbp-8* to form a positive feedback loop [53] and mitochondrial genes to induce lysosome–mitochondria crosstalk [55]. The activation of this lysosome–nucleus retrograde lipid signaling pathway by overexpressing *lipl-4* or *lbp-8* or by supplementing OEA promotes longevity, lipid catabolism, and redox homeostasis [53,55].

#### Lysosome–Nucleus Shuttling of TFEB and MITF-1

Unlike nuclear hormone receptors mostly residing in the nucleus, numerous TFs shuttle between the cytoplasm and the nucleus in response to different stimuli. A common mechanism is the sequestration of TFs in the cytoplasm until specific cues trigger their translocation to the nucleus and regulate the expression of their target genes accordingly. In particular, the TFs, TFEB and **Microphthalmia-associated transcription factor** (MITF)-1, can be recruited to the lysosome by interacting with active Rag GTPase heterodimers, leading to mTORC1-dependent inhibition of their activities (Figure 2) [56]. TFEB and MITF-1 both belong to the MITF subfamily of helix-loop-helix leucine zipper TFs that also includes TFE3 [57]. When nutrients are abundant, TFEB and MITF-1 are constantly cycling between the lysosome and the cytosol, and the lysosome-associated fraction can be phosphorylated by mTORC1, released back to the cytosol, and tethered with the **14-3-3** protein [56,58–60]. TFEB-14-3-3 tethering blocks the nuclear translocation of TFEB and, consequently, its activity.

Upon starvation or mTORC1 inactivation, dephosphorylated TFEB fails to tether with 14-3-3, translocates into the nucleus, and shows a stronger association with lysosomes [61–64]. Similarly, inactivation of mTORC1 can also lead to the accumulation of MITF-1 into the nucleus and at the lysosome [56]. In the nucleus, TFEB activates a series of target genes that control lysosomal



Trends in Biochemical Sciences

**Figure 2. Lysosome and Nucleus Interaction in Signaling and Transcription.** For the nucleus–lysosome interaction, the lysosomal–autophagic pathway is transcriptionally regulated in response to the nutrient status. Upon starvation, the transcription factors (TFEB/TFE3 (shown as TFEB), Forkhead box O3 (FOXO3), cAMP response element-binding protein (CREB)-CRTC2 peroxisome proliferator-activated receptor (PPAR) $\alpha$ , and p53 bind to the promoter region of lysosomal–autophagic genes and activate their expression. Meanwhile, ZKSCAN3 represses the expression of lysosomal–autophagic genes, as does farnesoid X receptor (FXR) by competing with PPAR $\alpha$  and dissociating the CREB-CRTC2 complex in well-fed conditions. For the lysosome–nucleus interaction, starvation can induce the nuclear translocation of fatty acid binding protein LBP-8, which binds oleoylethanolamine (OEA) and delivers it to the nuclear hormone receptor (NHR) complex NHR-49-NHR-80 to induce mitochondrial metabolic genes. By contrast, nutrient repletion results in lysosomal docking of mammalian target of rapamycin complex 1 (mTORC1), which phosphorylates TFEB/TFE3. The binding of phosphorylated TFEB/TFE3 with cytosolic 14-3-3 proteins prevents its nuclear translocation.

biogenesis and autophagy [15,65] (see ‘TFEB/TFE3 Transcription Factors’). Besides mTORC1, the phosphorylation and nuclear localization of TFEB can be regulated by several other kinases and phosphatases, including ERK2, protein phosphatase 2A (PP2A), and calcineurin, a  $\text{Ca}^{2+}$ -triggered serine-threonine phosphatase [61,66,67]. Interestingly, calcineurin is activated by  $\text{Ca}^{2+}$  release through the ion channel TRPML1, which is required for promoting  $\text{Ca}^{2+}$ -dependent lysosomal movement toward the perinuclear region upon nutrient deprivation [25]. Thus, lysosomal positioning might also couple with the modulation of TFEB activity.

### Nucleus–Lysosome Interaction in the Control of Lysosomal Adaptation

In supporting cellular homeostasis, not only do lysosomal signals actively influence nuclear transcription by altering lysosomal positioning and promoting nuclear translocation of chaperone proteins and TFs, but nuclear transcription also dynamically regulates genes involved in lysosomal biogenesis and functions and autophagy. Several TFs are implicated in this nuclear control of lysosomal adaptation to ensure that lysosomes can adjust their values and activities in the context of environment fluctuation (Figure 2).

### TFEB/TFE3 Transcription Factors

The discovery of the coordinated lysosomal expression and regulation (CLEAR) gene network and its responsible TF TFEB suggests that the lysosomal-autophagic pathway is under a transcriptional control [15,16]. Genes in the CLEAR network carry a CLEAR motif (TCACG) that is recognized and positively regulated by TFEB [15], and are extensively implicated in the lysosomal-autophagic process, such as autophagosome biogenesis, lysosome acidification, hydrolysis, ion homeostasis, and exocytosis [29,62,63]. In addition to TFEB, TFE3 also induces the expression of lysosomal biogenesis and autophagy genes via binding with the CLEAR motif [64].

TFEB and TFE3 act independently to some extent, and are both regulated by mTORC1, which phosphorylates them at critical serine residues, leading to 14-3-3 tethering and cytoplasmic retention [64,68,69]. Nutrient deprivation inhibits the mTORC1-dependent phosphorylation, promotes calcineurin-mediated dephosphorylation following lysosomal  $\text{Ca}^{2+}$  release through TRPML1, and drives the translocation of TFEB/TFE3 into the nucleus [61]. Transcriptional induction of TFEB/TFE3 target genes enhances lysosome biogenesis and autophagic flux, which enable cells and organisms to meet the catabolic need required for survival under starvation. Other kinases responsive to metabolic cues and growth factors, such as MAP4K3, ERK, and **glycogen synthase kinase 3** (GSK3), can also phosphorylate TFEB/TFE3 and inhibit their nuclear localization [65,70–73].

As a counterpart of TFEB, a zinc-finger TF harboring KRAB and SCAN domains (ZKSCAN3) has been reported to translocate into the nucleus when nutrients are available and represses a large set of genes related to the lysosomal-autophagic pathway [74]. Knockdown of ZKSCAN3 increases lysosome biogenesis and promotes autophagy in cells [74]. However, studies from ZKSCAN3-knockout mice showed the dispensable role of this TF in regulating lysosomal or autophagic gene expression [75]. It remains unclear what might cause this discrepancy, although one possible explanation is that ZKSCAN3 knockout over a long time leads to transcriptional adaptation that hinders its effects.

### FOXO Transcription Factors and p53

The class O of forkhead transcription factors (FOXO) are involved in regulating numerous cellular processes, including cell metabolism, cell cycle and proliferation, oxidative stress response, and longevity [76–78]. *C. elegans* and *Drosophila melanogaster* each have one FOXO, whereas mammals have four members, FOXO1, FOXO3, FOXO4, and FOXO6 [76,79,80]. AKT is the primary regulator of FOXO, phosphorylating it and causing its tethering with 14-3-3 and retention in the cytoplasm [81]. In muscle cells, FOXO3 is required for the transcriptional induction of autophagy-related genes [82]. More recently, analysis of ChIP-seq data showed that FOXO3 directly binds to, and regulates, a network of autophagy-related genes in adult neural stem and progenitor cells, where its activation promotes autophagic flux [83]. In addition to FOXO3, FOXO1 has been shown to induce autophagy upon oxidative stress or serum starvation in the human colon cancer cell line HCT116 and human cervical carcinoma cell line HeLa, although this was independent of its transcription activity [84]. Moreover, the expression of autophagy-related genes is reduced in the cartilage-specific triple-knockout mice of FOXO1, 3, and 4, but induced in chondrocytes overexpressing the constitutively active form of FOXO1 [85].

For FOXO3, although ChIP-seq analysis reveals its binding to many autophagy-related genes, several of those genes are not transcriptionally regulated by FOXO3, suggesting a co-factor function of FOXO3. In supporting this idea, a more recent study in *C. elegans* showed that TFEB/HLH-30 directly interacts with FOXO/DAF-16 by forming a combinatorial TF complex [86]. Further transcriptomic analysis indicated that these two TFs jointly regulate many target

genes involved in the longevity pathway [86]. Given the vital function of FOXO in multiple stress and metabolic processes, its coordination with TFEB adds another layer to the regulation of lysosomal adaptation.

In addition, FOXO3a itself is subject to transcriptional activation by p53, a key tumor suppressor [87,88]. p53 family members also upregulate the expression of other genes involved in the lysosomal-autophagic process, from upstream factors mediating AMPK activation and mTOR inhibition, core autophagy components, to lysosomal proteins [88–91]. By contrast, basal cytoplasmic p53 has been shown to repress autophagy in different cellular contexts, likely through transcription-independent mechanisms [90]. Thus, p53 regulates the lysosomal-autophagic process in a dual fashion, acting as both an inducer and a suppressor, which demands further investigation.

### Nuclear Hormone Receptors

Nuclear hormone receptors are a family of ligand-inducible TFs that are commonly activated by various lipid signals, and some have been linked with the transcriptional regulation of the lysosomal-autophagic process. In hepatic cells, farnesoid X receptor (FXR), a well-known bile acid receptor, is activated by feeding-associated increase in bile acids and transcriptionally represses lysosomal and autophagic genes. In one way, this repression is mediated through the interaction between FXR and **cAMP response element-binding protein** (CREB), which hinders the binding of CREB with its coactivator CRTC2. Upon starvation, FXR-mediated inhibition of the CREB-CRTC2 complex is removed, allowing the transcriptional induction of lysosomal and autophagic genes [92].

In another way, this repression is mediated through competition between FXR and PPAR $\alpha$  on the same binding motif in the promoter regions of lysosomal and autophagic genes. Fasting activates PPAR $\alpha$  to induce the transcription of lysosomal and autophagic gene in the liver, while feeding activates FXR to repress the transcription of the same group of genes [93]. Interestingly, TFEB itself is a transcription target of FXR and CREB [92], which in turn transcriptionally regulates PPAR $\alpha$  and its coactivator **PPAR gamma coactivator 1-alpha** (PGC1 $\alpha$ ) [65]. Thus, these two mechanisms might coordinate to enhance lysosomal adaptation in response to metabolic changes. At the same time, specific FABP can directly bind with PPAR $\alpha$  and regulate its transcriptional activity [94]. In addition, OEA is a well-known endogenous agonist of PPAR $\alpha$  [95], which suggests the role of lysosomal FABP and lipid signals in the transcriptional regulation of lysosomal adaptation.

### Concluding Remarks

Traditionally viewed as terminal degradative organelles in catabolic processes, the fundamental roles of lysosomes in nutrient sensing and metabolic signal transduction have been revealed over the past decade. A variety of sensors and transducers within the cell refine metabolic cues from lysosomes and launch signaling cascades to promote appropriate cellular responses. The crosstalk between the lysosome and the nucleus is a crucial part of this signaling orchestra and occurs at multiple layers, including modulation of lysosomal motility and positioning, shuttling of signaling factors between the two compartments, and transcriptional control of lysosomal adaptation, as reviewed in this article.

Moreover, accumulating evidence suggests that lysosomes widely interact and cooperate with other organelles in addition to the nucleus. For example, lysosome–ER contact has crucial roles in regulating lysosomal positioning, Ca<sup>2+</sup> homeostasis, and mTOR signaling [38,96,97]. Both ER and lysosomes are intracellular Ca<sup>2+</sup> stores. The release of lysosomal Ca<sup>2+</sup> is implicated in endocytic membrane trafficking and fusion, autophagy, lysosome exocytosis, and plasma

### Outstanding Questions

Other than nutrient deprivation, are there environmental cues that modulate lysosome motility and positioning?

How does lysosomal positioning regulate lysosomal signaling in addition to mTOR, and do lysosomes recruit different proteins to the surface at peripheral and perinuclear regions for these regulations?

What is the inter-relationship between lysosomal positioning, functional heterogeneity, and signaling transduction, and how can we apply our knowledge of lysosomal positioning from cell culture systems to *in vivo* organisms?

Does the retrograde movement of lysosomes facilitate lysosome–nucleus signaling communication and the transcriptional activation of lysosomal functions?

Do changes in lysosomal positioning associated with diseases contribute to alternations in lysosome–nucleus communication and the transcriptional control of lysosomal adaptation?

How can we improve current imaging methods for better *in vivo* visualization of lysosomal heterogeneity, dynamics, and interactions with other organelles?

How do different TFs coordinate to regulate lysosomal adaptation in response to different external and internal stimuli?

Is the lysosome–nucleus communication mechanism mediated by FABPs conserved in mammals? What factors recruit FABPs to the lysosome and facilitate its translocation to the nucleus? What lysosome transporters coordinate with FABPs to export signaling lipids, and specific nuclear hormone receptors act downstream of lysosomal FABPs?

Are there other lipid chaperone proteins and lipid signals mediating lysosome–nucleus retrograde signaling? Are there lipid chaperone proteins mediating lipid-related interorganelle communication beyond the lysosome and the nucleus?

Is there a variety of TFs that can be shuttled between the cytosol, lysosome, and nucleus in response to

membrane repair [98,99]. A recent study showed that the inositol 1,4,5-trisphosphate (IP3) receptor is activated by IP3 populated at the contact site between ER and lysosomes and regulates  $\text{Ca}^{2+}$  exchange between them [100]. In addition, lysosome–ER contact is vital to monitor and regulate cholesterol levels, and **oxysterol-binding protein** (OSBP) can function at lysosome–ER contact sites and transport cholesterol across the contact to activate mTORC1 [97]. Physical contact between lysosomes and mitochondria is also reported in healthy untreated Hela cells, mediated by Rab7 [101]. Upon recruitment of the Rab7 GTPase-activating protein **TBC1 domain family member 15** (TBCF1D15) to mitochondria by **mitochondria fission 1 protein** (FIS1), increased GTP hydrolysis releases the contact between lysosomes and mitochondria [101]. Unraveling how lysosomes are able to coordinately interact with different organelles and the physiological relevance underlying these interactions will be a challenging and promising direction for future research. Cutting-edge imaging techniques, such as lattice light-sheet microscopy, cryogenic super-resolution fluorescence microscopy coupled with focused ion beam scanning electron microscopy [102–106], are shedding new light on this area.

Most of our knowledge regarding the signaling role of lysosomes comes from studies in cell culture systems. In an organism, LSDs usually show a range of cell and organ specificity [107]. Although this specificity may be due to different cellular and/or metabolic demands in different cells and organs, the heterogeneity of lysosomes across different cell types could contribute to different pathologies of distinct organs associated with LSDs [36,40,41]. Therefore, it will be crucial to harness the power of tissue and/or cell type-specific proteomics and metabolomics of lysosomes and high-resolution, high-dynamics microscopic analyses of lysosomes to investigate their heterogeneity, complex interaction with other organelles, and cell-autonomous and nonautonomous regulatory mechanisms at the organism level. These systemic studies will provide new ways for improving lysosomal functions in various tissues and deepen our current understanding of the functional roles of lysosomes in health and diseases (see Outstanding Questions).

### Acknowledgments

We thank the NIH [grants R01AG045183 (M.C.W.), R01AT009050 (M.C.W.), R01AG062257 (M.C.W.), and DP1DK113644 (M.C.W.)], March of Dimes Foundation (M.C.W.), Welch Foundation (M.C.W.), for funding. We apologize to colleagues whose work we were not able to cite owing to space limitations.

### References

- Appelmans, F. *et al.* (1955) Tissue fractionation studies. 5. The association of acid phosphatase with a special class of cytoplasmic granules in rat liver. *Biochem. J.* 59, 438–445
- Mindell, J.A. (2012) Lysosomal acidification mechanisms. *Annu. Rev. Physiol.* 74, 69–86
- Saftig, P. *et al.* (2010) Lysosomal membrane proteins: life between acid and neutral conditions. *Biochem. Soc. Trans.* 38, 1420–1423
- Chapel, A. *et al.* (2013) An extended proteome map of the lysosomal membrane reveals novel potential transporters. *Mol. Cell. Proteomics* 12, 1572–1588
- Tooze, S.A. *et al.* (2014) Endocytosis and autophagy: exploitation or cooperation? *Cold Spring Harb. Perspect. Biol.* 6, a018358
- Rabinowitz, J.D. and White, E. (2010) Autophagy and metabolism. *Science* 330, 1344–1348
- Sorkin, A. and M. von Zastrow, M. (2009) Endocytosis and signalling: intertwining molecular networks. *Nat. Rev. Mol. Cell Biol.* 10, 609–622
- Saftig, P. and Klumperman, J. (2009) Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. *Nat. Rev. Mol. Cell Biol.* 10, 623–635
- Lloyd, J.B. (1996) Metabolite efflux and influx across the lysosome membrane. *Subcell. Biochem.* 27, 361–386
- Liu, B. *et al.* (2012) LAAT-1 is the lysosomal lysine/arginine transporter that maintains amino acid homeostasis. *Science* 337, 351–354
- Blott, E.J. and Griffiths, G.M. (2002) Secretory lysosomes. *Nat. Rev. Mol. Cell Biol.* 3, 122–131
- Rodríguez, A. *et al.* (1997) Lysosomes behave as  $\text{Ca}^{2+}$ -regulated exocytic vesicles in fibroblasts and epithelial cells. *J. Cell Biol.* 137, 93–104
- Jaiswal, J.K. *et al.* (2002) Membrane proximal lysosomes are the major vesicles responsible for calcium-dependent exocytosis in nonsecretory cells. *J. Cell Biol.* 159, 625–635
- Chieregatti, E. and Meldolesi, J. (2005) Regulated exocytosis: new organelles for non-secretory purposes. *Nat. Rev. Mol. Cell Biol.* 6, 181–187
- Sardiello, M. *et al.* (2009) A gene network regulating lysosomal biogenesis and function. *Science* 325, 473–477
- Perera, R.M. and Zoncu, R. (2016) The lysosome as a regulatory hub. *Annu. Rev. Cell Dev. Biol.* 32, 223–253
- Ballabio, A. and Bonifacino, J.S. (2020) Lysosomes as dynamic regulators of cell and organismal homeostasis. *Nat. Rev. Mol. Cell Biol.* 21, 101–118
- van Bergeijk, P. *et al.* (2016) Right time, right place: probing the functions of organelle positioning. *Trends Cell Biol.* 26, 121–134
- Pu, J. *et al.* (2016) Mechanisms and functions of lysosome positioning. *J. Cell Sci.* 129, 4329–4339
- Pu, J. *et al.* (2015) BORC, a multisubunit complex that regulates lysosome positioning. *Dev. Cell* 33, 176–188

different stimuli, and what are the molecular mechanisms underlying this dynamic regulation?

What are the roles of epigenetic factors in the fine-tuning of lysosomal adaptation to various environmental cues, and how do they cooperate with the various TFs involved?

At the organismal level, how heterogeneous are lysosomes across different cell types and tissues? What is the physiological and functional relevance of lysosomal heterogeneity, and how is it related to different pathologies of organs associated with lysosomal storage disorders, neurodegenerative diseases, and aging?

21. Pu, J. *et al.* (2017) A Regulator-BORC interaction controls lysosome positioning in response to amino acid availability. *J. Cell Biol.* 216, 4183–4197
22. Jordens, I. *et al.* (2001) The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein-dynactin motors. *Curr. Biol.* 11, 1680–1685
23. Rocha, N. *et al.* (2009) Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 Glued and late endosome positioning. *J. Cell Biol.* 185, 1209–1225
24. Vergarajauregui, S. *et al.* (2009) Identification of the penta-EF-hand protein ALG-2 as a Ca<sup>2+</sup>-dependent interactor of mucopolin-1. *J. Biol. Chem.* 284, 36357–36366
25. Li, X. *et al.* (2016) A molecular mechanism to regulate lysosome motility for lysosome positioning and tubulation. *Nat. Cell Biol.* 18, 404–417
26. Dong, X.P. *et al.* (2008) The type IV mucopolidiosis-associated protein TRPML1 is an endolysosomal iron release channel. *Nature* 455, 992–996
27. Dong, X.P. *et al.* (2010) PI(3,5)P<sub>2</sub> controls membrane trafficking by direct activation of mucopolin Ca(2+) release channels in the endolysosome. *Nat. Commun.* 1, 38
28. Bridges, D. *et al.* (2012) Phosphatidylinositol 3,5-bisphosphate plays a role in the activation and subcellular localization of mechanistic target of rapamycin 1. *Mol. Biol. Cell* 23, 2955–2962
29. Willett, R. *et al.* (2017) TFEB regulates lysosomal positioning by modulating TMEM55B expression and JIP4 recruitment to lysosomes. *Nat. Commun.* 8, 1580
30. Takemasu, S. *et al.* (2019) Phosphorylation of TMEM55B by Erk/MAPK regulates lysosomal positioning. *J. Biochem.* 166, 175–185
31. Wang, T. and Hong, W. (2002) Interorganellar regulation of lysosome positioning by the Golgi apparatus through Rab34 interaction with Rab-interacting lysosomal protein. *Mol. Biol. Cell* 13, 4317–4332
32. Stirling, G.P. *et al.* (2016) Folliculin directs the formation of a Rab34-RILP complex to control the nutrient-dependent dynamic distribution of lysosomes. *EMBO Rep.* 17, 823–841
33. Jongsma, M.L. *et al.* (2016) An ER-associated pathway defines endosomal architecture for controlled cargo transport. *Cell* 166, 152–166
34. Guardia, C.M. *et al.* (2016) BORC Functions upstream of kinesins 1 and 3 to coordinate regional movement of lysosomes along different microtubule tracks. *Cell Rep.* 17, 1950–1961
35. Rosa-Ferreira, C. and Munro, S. (2011) Arl8 and SKIP act together to link lysosomes to kinesin-1. *Dev. Cell* 21, 1171–1178
36. Korolchuk, V.I. *et al.* (2011) Lysosomal positioning coordinates cellular nutrient responses. *Nat. Cell Biol.* 13, 453–460
37. Raiborg, C. *et al.* (2015) Repeated ER-endosome contacts promote endosome translocation and neurite outgrowth. *Nature* 520, 234–238
38. Hong, Z. *et al.* (2017) PtdIns3P controls mTORC1 signaling through lysosomal positioning. *J. Cell Biol.* 216, 4217–4233
39. Nobukuni, T. *et al.* (2005) Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. *Proc. Natl. Acad. Sci. U. S. A.* 102, 14238–14243
40. Johnson, D.E. *et al.* (2016) The position of lysosomes within the cell determines their luminal pH. *J. Cell Biol.* 212, 677–692
41. Gowrishankar, S. and Ferguson, S.M. (2016) Lysosomes relax in the cellular suburbs. *J. Cell Biol.* 212, 617–619
42. Kim, J. and Guan, K.L. (2019) mTOR as a central hub of nutrient signalling and cell growth. *Nat. Cell Biol.* 21, 63–71
43. Savini, M. *et al.* (2019) Lysosomes: signaling hubs for metabolic sensing and longevity. *Trends Cell Biol.* 29, 876–887
44. Liu, G.Y. and Sabatini, D.M. (2020) mTOR at the nexus of nutrition, growth, ageing and disease. *Nat. Rev. Mol. Cell Biol.* 21, 183–203
45. Filippek, P.A. *et al.* (2017) LAMTOR/Regulator is a negative regulator of Arl8b- and BORC-dependent late endosomal positioning. *J. Cell Biol.* 216, 4199–4215
46. Jia, R. and Bonifacio, J.S. (2019) Lysosome positioning influences mTORC2 and AKT signaling. *Mol. Cell* 75, 26–38
47. Clippinger, A.J. and Alwine, J.C. (2012) Dynein mediates the localization and activation of mTOR in normal and human cytomegalovirus-infected cells. *Genes Dev.* 26, 2015–2026
48. Walton, Z.E. *et al.* (2018) Acid suspends the circadian clock in hypoxia through inhibition of mTOR. *Cell* 174, 72–87
49. Duffy, J. *et al.* (2017) Lipid metabolism, lipid signalling and longevity. In *Ageing: Lessons from C. elegans* (Olsen, A. and Gill, M.S., eds), pp. 307–329, Springer International Publishing
50. Thelen, A.M. and Zoucu, R. (2017) Emerging roles for the lysosome in lipid metabolism. *Trends Cell Biol.* 27, 833–850
51. Furuhashi, M. and Hotamisligil, G.S. (2008) Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nat. Rev. Drug Discov.* 7, 489–503
52. Tan, N.S. *et al.* (2002) Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription. *Mol. Cell Biol.* 22, 5114–5127
53. Folick, A. *et al.* (2015) Lysosomal signaling molecules regulate longevity in *Caenorhabditis elegans*. *Science* 347, 83–86
54. Tillman, M.C. *et al.* (2019) Structural characterization of life-extending *Caenorhabditis elegans* lipid binding protein 8. *Sci. Rep.* 9, 9966
55. Ramachandran, P.V. *et al.* (2019) Lysosomal signaling promotes longevity by adjusting mitochondrial activity. *Dev. Cell* 48, 685–696
56. Martina, J.A. and Puertollano, R. (2013) Rag GTPases mediate amino acid-dependent recruitment of TFEB and MITF to lysosomes. *J. Cell Biol.* 200, 475–491
57. Rehli, M. *et al.* (1999) Cloning and characterization of the murine genes for bHLH-ZIP transcription factors TFEB and TFEB reveal a common gene organization for all MIT subfamily members. *Genomics* 56, 111–120
58. Bronisz, A. *et al.* (2006) Microphthalmia-associated transcription factor interactions with 14-3-3 modulate differentiation of committed myeloid precursors. *Mol. Biol. Cell* 17, 3897–3906
59. Settembre, C. *et al.* (2012) A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. *EMBO J.* 31, 1095–1108
60. Roczniak-Ferguson, A. *et al.* (2012) The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Sci. Signal.* 5, ra42
61. Medina, D.L. *et al.* (2015) Lysosomal calcineurin signaling regulates autophagy through calcineurin and TFEB. *Nat. Cell Biol.* 17, 288–299
62. Medina, D.L. *et al.* (2011) Transcriptional activation of lysosomal exocytosis promotes cellular clearance. *Dev. Cell* 21, 421–430
63. Sbano, L. *et al.* (2017) TFEB-mediated increase in peripheral lysosomes regulates store-operated calcium entry. *Sci. Rep.* 7, 40797
64. Martina, J.A. *et al.* (2014) The nutrient-responsive transcription factor TFE3 promotes autophagy, lysosomal biogenesis, and clearance of cellular debris. *Sci. Signal.* 7, ra9
65. Settembre, C. *et al.* (2011) TFEB links autophagy to lysosomal biogenesis. *Science* 332, 1429–1433
66. Tong, Y. and Song, F. (2015) Intracellular calcium signaling regulates autophagy via calcineurin-mediated TFEB dephosphorylation. *Autophagy* 11, 1192–1195
67. Wang, W. *et al.* (2015) Up-regulation of lysosomal TRPML1 channels is essential for lysosomal adaptation to nutrient starvation. *Proc. Natl. Acad. Sci. U. S. A.* 112, E1373–E1381
68. Xu, Y. *et al.* (2019) YWHA/14-3-3 proteins recognize phosphorylated TFEB by a noncanonical mode for controlling TFEB cytoplasmic localization. *Autophagy* 15, 1017–1030
69. Martina, J.A. *et al.* (2012) mTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy* 8, 903–914
70. Marchand, B. *et al.* (2015) Glycogen synthase kinase-3 (GSK3) inhibition induces prosurvival autophagic signals in human pancreatic cancer cells. *J. Biol. Chem.* 290, 5592–5605
71. Hsu, C.L. *et al.* (2018) MAP4K3 mediates amino acid-dependent regulation of autophagy via phosphorylation of TFEB. *Nat. Commun.* 9, 942
72. Li, Y. *et al.* (2016) Protein kinase C controls lysosome biogenesis independently of mTORC1. *Nat. Cell Biol.* 18, 1065–1077
73. Li, L. *et al.* (2018) A TFEB nuclear export signal integrates amino acid supply and glucose availability. *Nat. Commun.* 9, 2685
74. Chauhan, S. *et al.* (2013) ZKSCAN3 is a master transcriptional repressor of autophagy. *Mol. Cell* 50, 16–28

75. Pan, H. *et al.* (2017) The role of ZKSCAN3 in the transcriptional regulation of autophagy. *Autophagy* 13, 1235–1238
76. Webb, A.E. and Brunet, A. (2014) FOXO transcription factors: key regulators of cellular quality control. *Trends Biochem. Sci.* 39, 159–169
77. Klotz, L.O. *et al.* (2015) Redox regulation of FoxO transcription factors. *Redox Biol.* 6, 51–72
78. Cheng, Z. (2019) The FoxO-autophagy axis in health and disease. *Trends Endocrinol. Metab.* 30, 658–671
79. Tissenbaum, H.A. (2018) DAF-16: FOXO in the Context of *C. elegans*. *Curr. Top. Dev. Biol.* 127, 1–21
80. Webb, A.E. *et al.* (2016) Characterization of the direct targets of FOXO transcription factors throughout evolution. *Aging Cell* 15, 673–685
81. Tzivion, G. *et al.* (2011) FoxO transcription factors; regulation by AKT and 14-3-3 proteins. *Biochim. Biophys. Acta* 1813, 1938–1945
82. Mammucari, C. *et al.* (2007) FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab.* 6, 458–471
83. Audesse, A.J. *et al.* (2019) FOXO3 directly regulates an autophagy network to functionally regulate proteostasis in adult neural stem cells. *PLoS Genet.* 15, e1008097
84. Zhao, Y. *et al.* (2010) Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity. *Nat. Cell Biol.* 12, 665–675
85. Matsuzaki, T. *et al.* (2018) FoxO transcription factors modulate autophagy and proteoglycan 4 in cartilage homeostasis and osteoarthritis. *Sci. Transl. Med.* 10, eaan0746
86. Lin, X.X. *et al.* (2018) DAF-16/FOXO and HLH-30/TFEB function as combinatorial transcription factors to promote stress resistance and longevity. *Nat. Commun.* 9, 4400
87. Renault, V.M. *et al.* (2011) The pro-longevity gene FoxO3 is a direct target of the p53 tumor suppressor. *Oncogene* 30, 3207–3221
88. Kenzelmann Broz, D. *et al.* (2013) Global genomic profiling reveals an extensive p53-regulated autophagy program contributing to key p53 responses. *Genes Dev.* 27, 1016–1031
89. Crighton, D. *et al.* (2006) DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* 126, 121–134
90. Tasdemir, E. *et al.* (2008) Regulation of autophagy by cytoplasmic p53. *Nat. Cell Biol.* 10, 676–687
91. Brady, O.A. *et al.* (2018) The transcription factors TFE3 and TFEB amplify p53 dependent transcriptional programs in response to DNA damage. *eLife* 7, e40856
92. Seok, S. *et al.* (2014) Transcriptional regulation of autophagy by an FXR-CREB axis. *Nature* 516, 108–111
93. Lee, J.M. *et al.* (2014) Nutrient-sensing nuclear receptors coordinate autophagy. *Nature* 516, 112–115
94. Hostettler, H.A. *et al.* (2009) L-FABP directly interacts with PPARalpha in cultured primary hepatocytes. *J. Lipid Res.* 50, 1663–1675
95. Fu, J. *et al.* (2003) Oleyethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. *Nature* 425, 90–93
96. Ganity, A.G. *et al.* (2016) The endoplasmic reticulum, not the pH gradient, drives calcium refilling of lysosomes. *eLife* 5, e15887
97. Lim, C.Y. *et al.* (2019) ER-lysosome contacts enable cholesterol sensing by mTORC1 and drive aberrant growth signalling in Niemann-Pick type C. *Nat. Cell Biol.* 21, 1206–1218
98. Li, P. *et al.* (2019) Lysosomal ion channels as decoders of cellular signals. *Trends Biochem. Sci.* 44, 110–124
99. Xu, H. and Ren, D. (2015) Lysosomal physiology. *Annu. Rev. Physiol.* 77, 57–80
100. Atakpa, P. *et al.* (2018) IP3 receptors preferentially associate with ER-lysosome contact sites and selectively deliver Ca<sup>2+</sup> to lysosomes. *Cell Rep.* 25, 3180–3193
101. Wong, Y.C. *et al.* (2018) Mitochondria-lysosome contacts regulate mitochondrial fission via RAB7 GTP hydrolysis. *Nature* 554, 382–386
102. Chen, B.C. *et al.* (2014) Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. *Science* 346, 1257998
103. Guo, Y. *et al.* (2018) Visualizing intracellular organelle and cytoskeletal interactions at nanoscale resolution on millisecond timescales. *Cell* 175, 1430–1442
104. Liu, Z. *et al.* (2015) Imaging live-cell dynamics and structure at the single-molecule level. *Mol. Cell* 58, 644–659
105. Valm, A.M. *et al.* (2017) Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature* 546, 162–167
106. Hoffman, D.P. *et al.* (2020) Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells. *Science* 367, eaaz5357
107. Schultz, M.L. *et al.* (2011) Clarifying lysosomal storage diseases. *Trends Neurosci.* 34, 401–410
108. Cuervo, A.M. and Dice, J.F. (1996) A receptor for the selective uptake and degradation of proteins by lysosomes. *Science* 273, 501–503
109. Chiang, H.L. *et al.* (1989) A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. *Science* 246, 382–385
110. Dice, J.F. (1990) Peptide sequences that target cytosolic proteins for lysosomal proteolysis. *Trends Biochem. Sci.* 15, 305–309
111. Oku, M. and Sakai, Y. (2018) Three distinct types of microautophagy based on membrane dynamics and molecular machineries. *Bioessays* 40, e1800008
112. Zhao, Y.G. and Zhang, H. (2019) Autophagosome maturation: an epic journey from the ER to lysosomes. *J. Cell Biol.* 218, 757–770
113. Nakatogawa, H. (2020) Mechanisms governing autophagosome biogenesis. *Nat. Rev. Mol. Cell Biol.* Published online May 5, 2020. <https://doi.org/10.1038/s41580-020-0241-0>
114. Parenti, G. *et al.* (2015) Lysosomal storage diseases: from pathophysiology to therapy. *Annu. Rev. Med.* 66, 471–486
115. Marques, A.R.A. and Saftig, P. (2019) Lysosomal storage disorders - challenges, concepts and avenues for therapy: beyond rare diseases. *J. Cell Sci.* 132, jcs221739
116. Dehay, B. *et al.* (2013) Lysosomal impairment in Parkinson's disease. *Mov. Disord.* 28, 725–732
117. Uddin, M.S. *et al.* (2019) Autophagic dysfunction in Alzheimer's disease: cellular and molecular mechanistic approaches to halt Alzheimer's pathogenesis. *J. Cell. Physiol.* 234, 8094–8112
118. Martin, D.D. *et al.* (2015) Autophagy in Huntington disease and huntingtin in autophagy. *Trends Neurosci.* 38, 26–35
119. Sharma, A. *et al.* (2016) Cerebrospinal fluid from sporadic amyotrophic lateral sclerosis patients induces mitochondrial and lysosomal dysfunction. *Neurochem. Res.* 41, 965–984
120. Lie, P.P.Y. and Nixon, R.A. (2019) Lysosome trafficking and signaling in health and neurodegenerative diseases. *Neurobiol. Dis.* 122, 94–105
121. Song, W. *et al.* (2013) TFEB regulates lysosomal proteostasis. *Hum. Mol. Genet.* 22, 1994–2009
122. Spannato, C. *et al.* (2013) Transcription factor EB (TFEB) is a new therapeutic target for Pompe disease. *EMBO Mol. Med.* 5, 691–706
123. Tsunemi, T. *et al.* (2012) PGC-1alpha rescues Huntington's disease proteotoxicity by preventing oxidative stress and promoting TFEB function. *Sci. Transl. Med.* 4, 142ra97
124. Martini-Stoica, H. *et al.* (2016) The autophagy-lysosomal pathway in neurodegeneration: a TFEB perspective. *Trends Neurosci.* 39, 221–234
125. Martini-Stoica, H. *et al.* (2018) TFEB enhances astroglial uptake of extracellular tau species and reduces tau spreading. *J. Exp. Med.* 215, 2355–2377
126. Polito, V.A. *et al.* (2014) Selective clearance of aberrant tau proteins and rescue of neurotoxicity by transcription factor EB. *EMBO Mol. Med.* 6, 1142–1160
127. Silvestrini, M.J. *et al.* (2018) Nuclear export inhibition enhances HLH-30/TFEB activity, autophagy, and lifespan. *Cell Rep.* 23, 1915–1921
128. Kimmelman, A.C. and White, E. (2017) Autophagy and tumor metabolism. *Cell Metab.* 25, 1037–1043
129. Perera, R.M. *et al.* (2019) MIT/TFE family of transcription factors, lysosomes, and cancer. *Annu. Rev. Cancer Biol.* 3, 203–222
130. Perera, R.M. *et al.* (2015) Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism. *Nature* 524, 361–365
131. Yang, S. *et al.* (2011) Pancreatic cancers require autophagy for tumor growth. *Genes Dev.* 25, 717–729

132. Kauffman, E.C. *et al.* (2014) Molecular genetics and cellular features of TFE3 and TFEB fusion kidney cancers. *Nat. Rev. Urol.* 11, 465–475
133. Calcagni, A. *et al.* (2016) Modelling TFE renal cell carcinoma in mice reveals a critical role of WNT signaling. *eLife* 5, e17047
134. Martins, R. *et al.* (2016) Long live FOXO: unraveling the role of FOXO proteins in aging and longevity. *Aging Cell* 15, 196–207
135. Lapiere, L.R. *et al.* (2013) The TFEB orthologue HLH-30 regulates autophagy and modulates longevity in *Caenorhabditis elegans*. *Nat. Commun.* 4, 2267
136. Reid, P.C. *et al.* (2003) Trafficking defects in endogenously synthesized cholesterol in fibroblasts, macrophages, hepatocytes, and glial cells from Niemann-Pick type C1 mice. *J. Lipid Res.* 44, 1010–1019
137. Kwiatkowska, K. *et al.* (2014) Visualization of cholesterol deposits in lysosomes of Niemann-Pick type C fibroblasts using recombinant perfringolysin O. *Orphanet J. Rare Dis.* 9, 64
138. Uusi-Rauva, K. *et al.* (2012) Neuronal ceroid lipofuscinosis protein CLN3 interacts with motor proteins and modifies location of late endosomal compartments. *Cell. Mol. Life Sci.* 69, 2075–2089
139. Erie, C. *et al.* (2015) Altered lysosomal positioning affects lysosomal functions in a cellular model of Huntington's disease. *Eur. J. Neurosci.* 42, 1941–1951
140. Wong, Y.C. and Holzbaur, E.L. (2014) The regulation of autophagosome dynamics by huntingtin and HAP1 is disrupted by expression of mutant huntingtin, leading to defective cargo degradation. *J. Neurosci.* 34, 1293–1305
141. Tamminen, P. *et al.* (2017) Impaired retrograde transport of axonal autophagosomes contributes to autophagic stress in Alzheimer's disease neurons. *eLife* 6, e21776
142. Gowrishankar, S. *et al.* (2015) Massive accumulation of luminal protease-deficient axonal lysosomes at Alzheimer's disease amyloid plaques. *Proc. Natl. Acad. Sci. U. S. A.* 112, E3699–E3708