Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Lysosomal signaling in control of degradation pathways

Lukas A Huber and David Teis

Autophagy mediates the (non-)selective bulk degradation of cytoplasm, protein aggregates, damaged organelles and certain pathogens. The endosomal membrane system uses multivesicular bodies (MVBs) to selectively deliver ubiquitinated membrane proteins together with extracellular components into lysosomes. Microautophagy (MA) and chaperone-mediated autophagy (CMA) additionally contribute to the selective delivery of cargo into lysosomes. The coordinated function of these lysosomal degradation pathways is essential to maintain cellular homeostasis. Their activity is controlled by mTOR (mammalian target of rapamycin) signaling and thus coupled to metabolic processes during cell growth. Here, we will discuss how TORC1 on lysosomes and TORC2 at the plasma membrane coordinate the different membrane biogenesis pathways with cargo selection, vesicle transport and fusion with lysosomes in response to intracellular and extracellular cues.

Address
Division of Cell Biology, Biocenter, Medical University of Innsbruck, Austria

Corresponding author: Teis, David (david.teis@i-med.ac.at)

Current Opinion in Cell Biology 2016, **39**:8–14

This review comes from a themed issue on **Cell regulation**

Edited by **Manuela Baccarini** and **Ivan Dikic**

For a complete overview see the Issue and the Editorial

Available online 28th January 2016

http://dx.doi.org/10.1016/j.ceb.2016.01.006

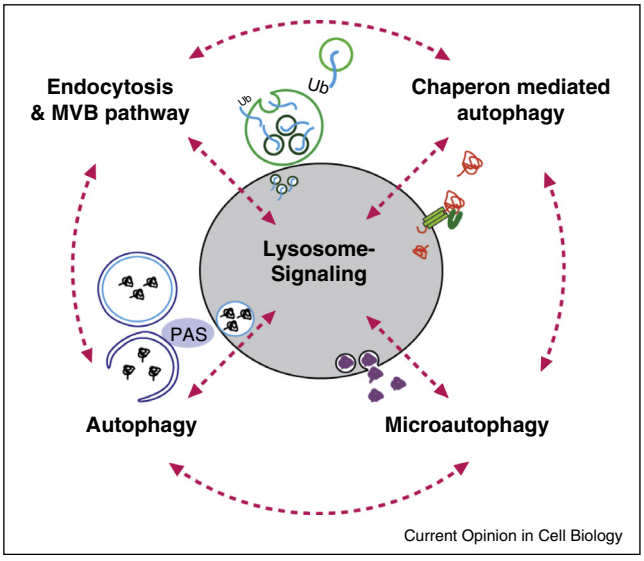
For a long time lysosomal degradation pathways have been considered as means to rid cells of waste products, simply required for the disposal and degradation of intra-cellular and extracellular macromolecules. Today, it has become clear that these catabolic pathways serve a much broader function than originally anticipated, including the regulation of cell signaling, metabolism and degradation of toxic protein aggregates and of damaged organelles as well as pathogen clearance. Their activity is tightly regulated and defects in each of these lysosomal degradation pathways can lead to metabolic disorders, cancer or neuro-degeneration [1,2]. The major goal of this review is to highlight our current understanding of how signaling from lysosomes triggers and coordinates different lysosomal degradation pathways and how they interact to maintain cellular homeostasis and organismal health (Figures 1 and 2).

Different vesicular and non-vesicular pathways target cargo to lysosomes for degradation (Figure 1). Autophagy delivers cytoplasmic material, damaged organelles, invading pathogens and protein aggregates into lysosomes [3]. Extracellular cargo and nutrients, together with components of the plasma membrane, integral membrane proteins and hydrolytic enzymes reach the lumen of lysosomes through the endosomal system via the multivesicular body (MVB) pathway [4]. Together, the MVB pathway and autophagy probably deliver the lion's share of cellular material to lysosomes. In addition, microautophagy (MA) and chaperone mediated autophagy (CMA) deliver cargo selectively into lysosomes [5]. The function of these lysosomal degradation pathways requires complex molecular machineries. The autophagy (ATG)-core machinery and the endosomal sorting complexes required for transport (ESCRT) couple (selective) cargo sorting to elaborate membrane biogenesis reactions to form transport intermediates [6,7]. (Endosomal) MA also relies on the ESCRT machinery for membrane remodeling [8]. Heterotypic membrane fusion of MVBs or autophagosomes with lysosomes (or with each other) finally delivers the cargo into the lumen of lysosomes. Cytoplasmic proteins degraded by CMA take a non-vesicular route into lysosomes and are directly imported across the limiting membrane of lysosomes. We are just beginning to understand how the individual pathways function. Yet, it is unclear how they are triggered and how these processes are coordinated with each other.

Signaling pathways central for cell growth and survival adjust the flux of cargo and regulate the biogenesis of lysosomal degradation pathways (Figure 2). The most prominent example is mTOR signaling, which integrates signaling from nutrients, growth factors, and energy availability [9]. mTOR exists in two distinct complexes, mTORC1 and mTORC2. They are found in various subcellular locations [10] and fulfill different tasks. Amongst others, mTORC1 signaling on late endosomes (LE)/lysosomes and mTORC2 at the PM and on lysosomes also seem to control cargo selection, membrane biogenesis and trafficking in different lysosomal degradation pathways.

Activation of mTORC1 on the surface of MVBs/lysosomes by Rheb-GTP is a complex multi-step process and additionally requires the RagA-D family of GTPases. They form obligatory dimers (e.g. RagA/C) and in their GTP bound form they recruit mTORC1 to MVBs/lysosomes. Rag-GTP loading is mediated by their guanine exchange factor (GEF), the LE/lysosomal adaptor and

Figure 1



Regulation of lysosomal degradation pathways. Autophagy, endocytosis and the MVB pathway, chaperone mediated autophagy (CMA) and microautophagy (MA) are depicted. Regulation by lysosomal signaling (see also Figure 2) and potential cross-talks are indicated.

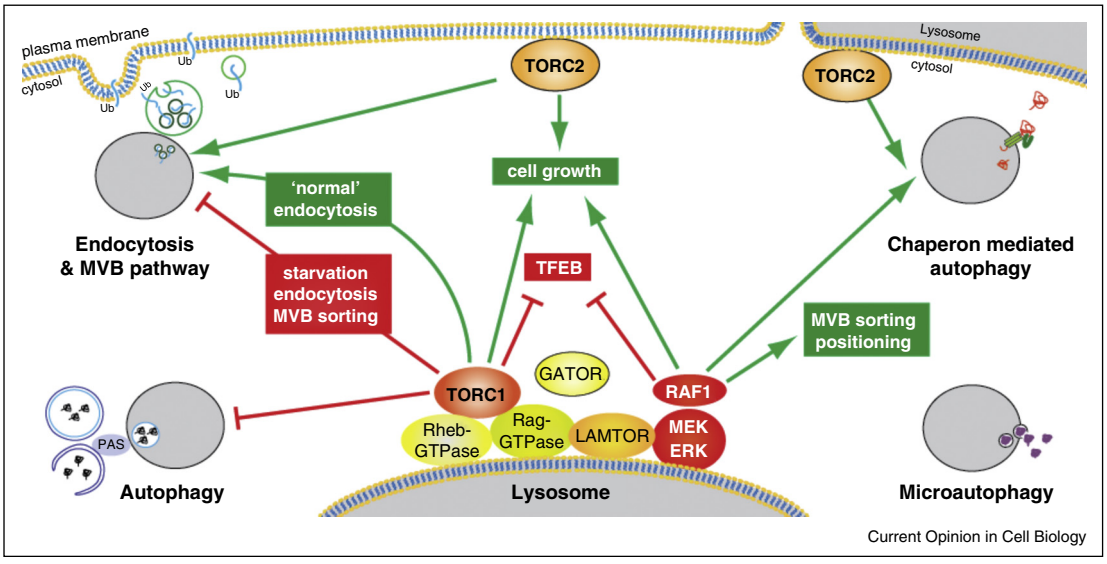
MAPK and mTOR activator complex (LAMTOR) also known as Ragulator [11–13].

LAMTOR is a pentameric complex that is anchored by one of its subunits to LE/lysosomes [13,14]. The GEF activity of LAMTOR toward the Rag-GTPases and hence mTORC1 activation on lysosomes is regulated by the interaction with a lysosomal nutrient transporter (SLC38A9) and the v-ATPase [15,16••,17••]. In addition, LAMTOR functions as a scaffold complex for ERK signaling in response to growth factors [11,14,18,19]. LAMTOR mediated mTORC1 and ERK signaling control the nuclear translocation of the transcription factor, TFEB [20,21,22]. mTORC1 signaling appears to play the predominate role in regulating the nuclear translocation of TFEB in response to nutrient deprivation or lysosomal stress/dysfunction. The role of ERK signaling in TFEB regulation is less clear. Just as important as the activation of TOR signaling is its inactivation [23•]. The Rag-GTPases are inactivated by multisubunit complexes named SEA/GATOR that function as GTPase activating proteins (GAPs) [24••,25,26•,27].

In line with the central role of mTOR and MAPK signaling in cell growth, LAMTOR and GATOR are required for tissue homeostasis *in vivo* and embryonic development [14,19,24••,28•]. Maybe more surprisingly, LAMTOR also controls several aspects of LE biogenesis. Its function is required for growth factor receptor degradation, lysosomal positioning through transport along microtubules and for the formation of recycling tubes on late LE/lysosomes [19,29,30•]. In addition, both LAMTOR and GATOR regulate autophagy. Despite their essential cellular functions and their role in tissue homeostasis, little is known about how LAMTOR and GATOR function, how they are regulated and how they mechanistically contribute to mTORC1 activation on lysosomes.

Much less is known about the mechanism resulting in TORC2 signaling at the PM, but TORC2 can be

Figure 2



Schematic presentation of lysosomal signaling complexes and their roles in lysosomal degradation pathways.

activated in response to membrane stress and contributes to endocytosis and autophagy [31]. The balance between mTORC2 and the phosphatase PHLPP1 has been shown recently to control Akt signaling on lysosomes which impacts on CMA [32•].

Overall it appears that cargo transport via the MVB pathway, MA/CMA and autophagy can be triggered by the modulation of lysosomal mTOR signaling. How TOR signaling orchestrates and triggers the activity of lysosomal degradation pathways is not clear.

Autophagy. Macro-autophagy (hereafter autophagy) engulfs cytoplasmic cargo inside a double-membrane organelle (Figure 1). These so-called autophagosomes form *de novo* at the preautophagosomal structure (PAS). Typically, there is a single PAS near or at the vacuolar membrane in yeast and autophagosomes are formed throughout the cytoplasm of mammalian cells at so far undefined sites. Autophagy is induced by various cellular stresses, including nutrient limitation, the accumulation of misfolded proteins (aggrephagy), defective mitochondria (mitophagy), damaged DNA as well as invading pathogenes (xenophagy). While starvation-induced autophagy sequesters bulk cytoplasm in autophagosomes in a rather unspecific manner [33], it has become clear that autophagy can be highly selective with regard to the cargo material that is captured within autophagosomes.

Autophagy proceeds through a continuous maturation process with several defined steps: first, induction; second, isolation membrane nucleation; third, isolation membrane expansion coupled to cargo capture; fourth, isolation membrane closure and finally, fusion with the lytic compartment. During starvation, mTORC1 inactivation is required for the induction of autophagy.

The autophagic machinery consists of at least 36 genes (Atg1–Atg36) that contribute to one or more steps of the process. The precise mechanism of action of the autophagic machinery is largely enigmatic. Seventeen of the 36 Atg genes comprise the autophagic core machinery that is essential for most types of autophagy [34]. The core machinery can be subdivided into several functional groups [6]. Among these, the Atg1/ULK1 kinase complex is essential for the induction of autophagy [35]. The Atg1/ULK1 kinase complex is under the control of mTORC1 and regulates the traffic of the transmembrane protein Atg9 in small, possibly Golgi-derived vesicles to and from the PAS, the site of autophagosome biogenesis. Atg6/Beclin and Atg14 are part of the PI3K complex I (composed of the Vps34, Vps15, Atg14 and Beclin subunits), required for vesicle nucleation at the PAS [36,37]. The molecular mechanisms of PAS assembly are still unclear. Finally, the Atg8 and Atg12 ubiquitin-like conjugation systems are essential for isolation membrane expansion and closure. Atg8-family proteins are conjugated to phosphatidylethanolamine, a process mediated by Atg3 and Atg7 [38]. Atg12 is conjugated to Atg5 [39]. The Atg12–Atg5 conjugate subsequently interacts with Atg16 to support isolation membrane elongation and closure [40]. During selective types of autophagy, receptor proteins simultaneously interact with cargo material and Atg8-family proteins on the isolation membrane and thereby confer selectivity to autophagosome formation [41].

Once their biogenesis is complete, mature autophagosomes fuse either directly with lysosomes or first with MVBs to form amphisomes [42] that later fuse with lysosomes. Inside lysosomes, autophagic cargo is degraded and amino acids, lipids and glucose are transported across the vacuolar membrane back to the cytoplasm where they are recycled by cellular metabolism. Autophagy cooperates with the Ubiquitin Proteasome System (UPS) in the degradation of cytosolic proteins to supply amino acids to the cell [43,44]. How autophagy cooperates with other lysosomal degradation pathways is less clear.

Chaperone-mediated autophagy (CMA) is a pathway for delivery of substrates into the lysosomes that does not involve vesicular intermediates, in contrast to autophagy (Figure 1). CMA substrates contain a pentapeptide (KFERQ-like) motif that is recognized by the chaperone heat shock cognate protein 70 (Hsc70). Binding to Hsc70 mediates the translocation of substrates into the lysosomal lumen, which requires the oligomerisation of the lysosomal receptor lysosome-associated membrane protein type 2A (Lamp2A) [45]. CMA can also modulate, compete and cooperate with the UPS for the degradation of short-lived proteins [46]. Thus, CMA plays a central role in the control of protein homeostasis and appears to be regulated by the lysosomal mTORC2/PHLPP1/Akt signaling axis [32•].

The multivesicular body (MVB) pathway transports endocytic and biosynthetic cargo into lysosomes (Figure 1). Endocytic cargo includes nutrient transporters, growth-factor receptor–ligand complexes, lipids and extracellular material and pathogens. The majority of these substances are not degraded but recycled back to the PM; only a small portion is delivered into lysosomes. Diverting membrane components into lysosomes requires stringent selection mechanisms that are coupled to MVB biogenesis. MVBs are specialized endosomes that are filled with small intraluminal vesicles (ILVs) with a defined diameter (25 nm in yeast, 50 nm in mammalian cells). The ILVs are the defining feature of these otherwise morphologically pleiotropic endocytic organelles. Only membrane proteins and lipids that are sorted into ILVs will be degraded inside lysosomes.

The significance of the MVB pathway was first appreciated when the activated epidermal growth factor receptor

(EGFR) was detected inside ILVs on its way into lysosomes [47]. Since then, it became clear that endocytosis and the MVB pathway contribute to the regulation of numerous cellular signaling pathways and determine how cells sense, respond and adapt to extracellular cues.

The down-regulation of the activated EGFR begins at the PM. Ligand binding not only activates mitogenic (Ras-RAF-MAPK) and pro-survival (Akt) signaling, but also results in ubiquitination of the activated receptor. Ubiquitin acts as the molecular tag that links the activated EGFR to the endocytic machinery. Ubiquitination and endocytosis of the activated EGFR are well-characterized processes [48]. In contrast, the events and molecular mechanisms leading to the ubiquitination and endocytosis of most membrane proteins, including the majority of nutrient transporters, are not clear.

On endosomes, only ubiquitinated membrane proteins enter the MVB pathway. All non-ubiquitinated membrane proteins are recycled back to the PM or to the Golgi. The biogenesis of MVBs is initiated by key effectors of the small GTPase Rab5 on early endosomes (EE), including the PI3K complex II (composed of the Vps34, Vps15, Vps38 and Beclin) [49,50]. In turn, phosphatidylinositol 3-phosphate (PI(3)P) and ubiquitinated membrane proteins [51] initiate the recruitment of the ESCRT machinery to endosomes, which coordinates sorting of ubiquitinated membrane proteins with MVB biogenesis [52–54]. ESCRT-0, ESCRT-I and ESCRT-II directly bind to ubiquitinated membrane proteins, while ESCRT-III and Vps4 drive membrane-remodeling reactions that result in ILV biogenesis [55•,56]. At the end of this process, the ESCRT machinery has sorted all ubiquitinated membrane proteins from the limiting membrane of MVBs into ILVs.

Also cytoplasmic proteins can be selectively packaged into ILVs in a process termed endosomal microautophagy (MA) in yeast and mammalian cells. In mammalian cells MA not only requires the ESCRT system, but also Hsc70, which mediates cargo selectivity. Hsc70 bind to endosomal membranes and interacts with cytoplasmic proteins containing KFERQ-motifs (similar to CMA) and thereby helps to package them into ILVs [8]. In the yeast *Schizosaccharomyces pombe*, a specific receptor (Nbr1) mediates the sorting of two cytosolic hydrolases via the ESCRT dependent MVB pathway into lysosomes [57].

Before MVBs can fuse with lysosomes, they mature from EE into LE, which requires a series of defined steps. These include first, switching from Rab5 to Rab7; second, conversion of PI(3)P to PI(3,5)P2; third, acidification and finally, changes in fusion specificity. At the end of this complex process, LE/MVBs are filled with cargo-laden ILVs, carry Rab7, PI(3,5)P2 and specific tethering (CORVET, HOPS) complexes and defined SNAREs (e.g. Stx7, Stx8, VTI1b and Vamp7) to promote fusion with lysosomes [4]. How these maturation steps are timed and regulated to prevent premature fusion with lysosomes is not well understood.

EE, recycling endosomes and MVBs contribute to autophagy, either as a membrane source of phagophore growth [58] or later when mature autophagosomes fuse with MVBs to form amphisomes [42] before they reach lysosomes. How these processes are regulated and whether amphisome formation is the default pathway to target autophagosomes to lysosomes is not clear.

Cooperation of lysosomal degradation pathways takes place at many different levels. Recently it has become clear that endocytosis of growth factor receptors and nutrient transporters not only occurs upon ligand binding under nutrient rich conditions, but is coordinated with a more prominent autophagic response upon TORC1 inactivation and/or nutrient limitation. Withdrawal of interleukin-3 results in endocytosis of several nutrient transporters (GLUT1, LAT1, Transferrin-Receptor and LDL-Receptor) and their subsequent lysosomal degradation via the MVB pathway [59,60]. Serum starvation induces endocytosis of the inactive EGFR, which, once on endosomes, contributes to the induction of autophagy by activation of the PI3K complex I [61]. In response to starvation, mTORC1 and ERK inactivation also promotes the nuclear translocation of MiT/TFE transcription factors (MITF, TFEB and TFE3). They induce the transcription of genes required for endo/lysosomal biogenesis and autophagy core components and thereby activate the so-called Coordinated Lysosomal Expression and Regulation (CLEAR) gene network [22,62]. In addition ZKSCAN3 and other transcriptional responses are critical to transcriptionally regulate the magnitude of autophagy [63]. Translating this transcriptional response into an efficient boost of lysosomal activity requires ongoing protein synthesis during nutrient limitation. In yeast this is achieved by massive starvation-induced endocytosis and membrane protein degradation in lysosomes via the MVB pathway. This supplies intracellular amino acids sufficient for continuous protein synthesis to boost the catabolic activity of lysosomes for the efficient degradation of autophagic cargo. In this way, starvation-induced endocytosis and autophagy cooperate to enter a stable G1/G0 quiescent state and thereby ensure cell survival upon nutrient limitation [64••]. Additionally, MA could use the ESCRT machinery during MVB biogenesis for the selective degradation of cytoplasmic proteins [8]. Overall, it is becoming clear that the MVB pathway plays a central role in cellular homeostasis that is coordinated with the larger autophagic response to starvation [64••], but the underlying molecular mechanisms have not been identified.

It is obvious that the catabolic activity of lysosomes is a prerequisite for the efficient function of each lysosomal degradation pathway and that most lysosomal degradation

pathways also deliver lysosomal enzymes and thereby, at least in part, contribute to lysosomal biogenesis.

Several key regulators of the endosomal system and autophagy also function in both pathways. Phosphatidyl-Inositol-3-Phosphate (PI(3)P) is essential for the biogenesis of EE, MVBs and autophagosomes [36]. The PI3K complex I is required in the early steps of autophagy for the recruitment of essential autophagic proteins [65]. On endosomes, PI(3)P is generated by the PI3K complex II [66,67]. Essential effectors of the MVB pathway (ESCRT complexes) bind to PI(3)P on endosomes [49]. Similarly PI(3,5)P2 is required for both autophagy and endosomal maturation [68,69]. Mounting evidence suggests that key regulators of endosomal transport contribute essentially to the biogenesis and/or the transport of autophagosomes. Prominent examples are Rab5 and Rab7, which have clearly defined functions in the endo-lysosomal system but additionally are required for autophagy [70–72] and/or the biogenesis of lysosomes. The same is true for their down-stream effectors such as different tethering complexes and SNAREs (e.g.: Vamp7, 8, Stx13, 17) that regulate fusion with lysosomes [73].

The ESCRT machinery itself was also found to be required for later steps in autophagy, possibly by regulating the formation of functional amphisomes and/or their fusion with lysosomes [74,75]. Alix, for instance, is a protein that associates with the ESCRT machinery and is required for MVB biogenesis and also interacts with Atg12–Atg3 of the autophagic core machinery. CMA can be activated to compensate for impaired autophagy via the up regulation of Lamp2a and changes in the abundance of lysosomal Hsc70 [76]. In turn, CMA down regulation is compensated by enhanced autophagy activity [77]. There are many more examples, but clear molecular mechanisms have been provided only for few.

Conclusion

In the past years it has become clear that lysosomal signaling controls lysosomal degradation pathways. The next challenge will be to gain mechanistic insight and more comprehensive understanding of how these processes engage with each other and are coordinated to ensure cellular homeostasis and prevent pathological changes. New insight may provide ideas and novel targets for therapeutic intervention and for the treatment of human diseases driven by defects in lysosomal signaling and degradation pathways.

Acknowledgements

The work was funded by HFSP-CDA-00001/2010-C, Austrian Science Fund (FWF), Y444-B12 to DT, FWF P 26682 to LAH and MCBO (W1101), SFB021 (F21) to LAH and DT. We would like to thank Claudine Kraft and Sascha Martens for comments and suggestions.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Settembre C, Fraldi A, Medina DL, Ballabio A: **Signals from the lysosome: a control centre for cellular clearance and energy metabolism**. *Nat Rev Mol Cell Biol* 2013, **14**:283-296.

2. Rusten TE, Simonsen A: **ESCRT functions in autophagy and associated disease**. *Cell Cycle* 2008, **7**:1166-1172.

3. Yang Z, Klionsky DJ: **Eaten alive: a history of macroautophagy**. *Nat Cell Biol* 2010, **12**:814-822.

4. Huotari J, Helenius A: **Endosome maturation**. *EMBO J* 2011, **30**:3481-3500.

5. Massey AC, Kaushik S, Cuervo AM: **Lysosomal chat maintains the balance**. *Autophagy* 2006, **2**:325-327.

6. Kraft C, Martens S: **Mechanisms and regulation of autophagosome formation**. *Curr Opin Cell Biol* 2012, **24**:496-501.

7. Schmidt O, Teis D: **The ESCRT machinery**. *Curr Biol: CB* 2012, **22**:R116-R120.

8. Sahu R, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, Potolicchio I, Nieves E, Cuervo AM, Santambrogio L: **Microautophagy of cytosolic proteins by late endosomes**. *Dev Cell* 2011, **20**:131-139.

9. Dibble CC, Manning BD: **Signal integration by mTORC1 coordinates nutrient input with biosynthetic output**. *Nat Cell Biol* 2013, **15**:555-564.

10. Betz C, Hall MN: **Where is mTOR and what is it doing there?** *J Cell Biol* 2013, **203**:563-574.

11. Teis D, Wunderlich W, Huber LA: **Localization of the MP1-MAPK scaffold complex to endosomes is mediated by p14 and required for signal transduction**. *Dev Cell* 2002, **3**:803-814.

12. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM: **Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids**. *Cell* 2010, **141**:290-303.

13. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM: **Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1**. *Cell* 2012, **150**:1196-1208.

14. Nada S, Hondo A, Kasai A, Koike M, Saito K, Uchiyama Y, Okada M: **The novel lipid raft adaptor p18 controls endosome dynamics by anchoring the MEK-ERK pathway to late endosomes**. *EMBO J* 2009, **28**:477-489.

15. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM: **mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase**. *Science* 2011, **334**:678-683.

16. •• Rebsamen M, Pochini L, Stasyk T, de Araujo MEG, Galluccio M, Kandasamy RK, Snijder B, Fauster A, Rudashevskaya EL, Bruckner M *et al.*: **SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1**. *Nature* 2015, **519**:477-481.
SLC38A9 is the first component of this complex shown to physically engage amino acids, suggesting a role at the core of the amino acid sensing mechanism.

17. •• Wang S, Tsun ZY, Wolfson RL, Shen K, Wyant GA, Plovanich ME, Yuan ED, Jones TD, Chantranupong L, Comb W *et al.*: **Metabolism. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1**. *Science* 2015, **347**:188-194.
The authors identify SLC38A9 upstream of the Rag GTPases one candidate for being an arginine sensor for the mTORC1 pathway.

18. Wunderlich W, Fialka I, Teis D, Alpi A, Pfeifer A, Parton RG, Lottspeich F, Huber LA: **A novel 14-kilodalton protein interacts with the mitogen-activated protein kinase scaffold mp1 on a**

late endosomal/lysosomal compartment. *J Cell Biol* 2001, **152**:765-776.

19. Teis D, Taub N, Kurzbauer R, Hilber D, de Araujo ME, Erlacher M, Offterdinger M, Villunger A, Geley S, Bohn G *et al.*: **p14-MP1-MEK1 signaling regulates endosomal traffic and cellular proliferation during tissue homeostasis**. *J Cell Biol* 2006, **175**:861-868.

20. Roczniak-Ferguson A, Petit CS, Froehlich F, Qian S, Ky J, Angarola B, Walther TC, Ferguson SM: **The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis**. *Sci Signal* 2012, **5**:ra42.

21. Settembre C, Zoncu R, Medina DL, Vetrini F, Erdin S, Erdin S, Huynh T, Ferron M, Karsenty G, Vellard MC *et al.*: **A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB**. *EMBO J* 2012, **31**:1095-1108.

22. Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, Erdin SU, Huynh T, Medina D, Colella P *et al.*: **TFEB links autophagy to lysosomal biogenesis**. *Science* 2011, **332**:1429-1433.

23. • Demetriades C, Doumpas N, Teleman AA: **Regulation of TORC1 in response to amino acid starvation via lysosomal recruitment of TSC2**. *Cell* 2014, **156**:786-799.
TSC2 is recruited by the Rag GTPases to lysosomes upon amino acid starvation and is required for complete inactivation of TORC1 upon amino acid starvation.

24. •• Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson M, Sabatini DM: **A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1**. *Science* 2013, **340**:1100-1106.
Identification of the octomeric GATOR complex as a critical regulator of the pathway that signals amino acid sufficiency to mTORC1. The GATOR1 subcomplex has GAP activity for RagA and RagB and its loss, for example, in tumor, makes mTORC1 signaling insensitive to amino acid deprivation.

25. Tsun ZY, Bar-Peled L, Chantranupong L, Zoncu R, Wang T, Kim C, Spooner E, Sabatini DM: **The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1**. *Mol Cell* 2013, **52**:495-505.

26. • Panchaud N, Peli-Gulli MP, De Virgilio C: **Amino acid deprivation inhibits TORC1 through a GTPase-activating protein complex for the Rag family GTPase Gtr1**. *Sci Signal* 2013, **6**:ra42.
The Iml1-Npr2-Npr3 complex may play an evolutionarily conserved role in suppressing Rag-mediated activation of TORC1, thereby contributing to the suppression of human tumor formation.

27. Panchaud N, Peli-Gulli MP, De Virgilio C: **SEACing the GAP that nEGOCiates TORC1 activation: Evolutionary conservation of Rag GTPase regulation**. *Cell Cycle* 2013, **12**.

28. • Scheffler JM, Sparber F, Tripp CH, Herrmann C, Humenberger A, Blitz J, Romani N, Stoitzner P, Huber LA: **LAMTOR2 regulates dendritic cell homeostasis through FLT3-dependent mTOR signalling**. *Nat Commun* 2014, **5**:5138.
Conditional ablation of *LAMTOR2* in dendritic cells (DC) results in an increased downstream activation of the AKT/mTOR signalling pathway and subsequently to a massive expansion of conventional DCs and plasmacytoid DCs in ageing mice.

29. Vogel GF, Ebner HL, de Araujo ME, Schmiedinger T, Eiter O, Pircher H, Gutleben K, Witting B, Teis D, Huber LA *et al.*: **Ultrastructural morphometry points to a New Role for LAMTOR2 in regulating the endo/lysosomal system**. *Traffic* 2015, **16**:617-634.

30. • Schiefermeier N, Scheffler JM, de Araujo ME, Stasyk T, Yordanov T, Ebner HL, Offterdinger M, Munck S, Hess MW, Wickstrom SA *et al.*: **The late endosomal p14-MP1 (LAMTOR2/3) complex regulates focal adhesion dynamics during cell migration**. *J Cell Biol* 2014, **205**:525-540.
This work demonstrates that LAMTOR2/3-positive endosomes are mobile signaling platforms that can move to the cell periphery along microtubules (MTs) in a kinesin1-dependent and Arl8b-dependent manner. There they specifically target FAs to regulate FA turnover.

31. Berchtold D, Piccolis M, Chiaruttini N, Riezman I, Riezman H, Roux A, Walther TC, Loewith R: **Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis**. *Nat Cell Biol* 2012, **14**:542-547.

32. • Arias E, Koga H, Diaz A, Mocholi E, Patel B, Cuervo AM: **Lysosomal mTORC2/PHLPP1/Akt regulate chaperone-mediated autophagy**. *Mol Cell* 2015, **59**:270-284.
This work shows that the mTORC2 regulates CMA on lysosomes.

33. Baba M, Osumi M, Scott SV, Klionsky DJ, Ohsumi Y: **Two distinct pathways for targeting proteins from the cytoplasm to the vacuole/lysosome**. *J Cell Biol* 1997, **139**:1687-1695.

34. Xie Z, Klionsky DJ: **Autophagosome formation: core machinery and adaptations**. *Nat Cell Biol* 2007, **9**:1102-1109.

35. Matsuura A, Tsukada M, Wada Y, Ohsumi Y: **Apg1p, a novel protein kinase required for the autophagic process in *Saccharomyces cerevisiae***. *Gene* 1997, **192**:245-250.

36. Simonsen A, Tooze SA: **Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes**. *J cell Biol* 2009, **186**:773-782.

37. Itakura E, Kishi C, Inoue K, Mizushima N: **Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG**. *Mol Biol Cell* 2008, **19**:5360-5372.

38. Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, Mizushima N, Tanida I, Kominami E, Ohsumi M *et al.*: **A ubiquitin-like system mediates protein lipidation**. *Nature* 2000, **408**:488-492.

39. Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, Klionsky DJ, Ohsumi M, Ohsumi Y: **A protein conjugation system essential for autophagy**. *Nature* 1998, **395**:395-398.

40. Mizushima N, Noda T, Ohsumi Y: **Apg16p is required for the function of the Apg12p–Apg5p conjugate in the yeast autophagy pathway**. *EMBO J* 1999, **18**:3888-3896.

41. Johansen T, Lamark T: **Selective autophagy mediated by autophagic adapter proteins**. *Autophagy* 2011, **7**:279-296.

42. Stromhaug PE, Seglen PO: **Evidence for acidity of prelysosomal autophagic/endocytic vacuoles (amphisomes)**. *Biochem J* 1993, **291(Pt 1)**:115-121.

43. Vabulas RM, Hartl FU: **Protein synthesis upon acute nutrient restriction relies on proteasome function**. *Science* 2005, **310**:1960-1963.

44. Suraweera A, Munch C, Hanssum A, Bertolotti A: **Failure of amino acid homeostasis causes cell death following proteasome inhibition**. *Mol Cell* 2012, **48**:242-253.

45. Arias E, Cuervo AM: **Chaperone-mediated autophagy in protein quality control**. *Curr Opin Cell Biol* 2011, **23**:184-189.

46. Park C, Cuervo AM: **Selective autophagy: talking with the UPS**. *Cell Biochem Biophys* 2013, **67**:3-13.

47. Haigler HT, McKanna JA, Cohen S: **Rapid stimulation of pinocytosis in human carcinoma cells A-431 by epidermal growth factor**. *J Cell Biol* 1979, **83**:82-90.

48. Tomas A, Futter CE, Eden ER: **EGF receptor trafficking: consequences for signaling and cancer**. *Trends Cell Biol* 2014, **24**:26-34.

49. Simonsen A, Lippe R, Christoforidis S, Gaullier JM, Brech A, Callaghan J, Toh BH, Murphy C, Zerial M, Stenmark H: **EEA1 links PI(3)K function to Rab5 regulation of endosome fusion**. *Nature* 1998, **394**:494-498.

50. Zeigerer A, Gilleron J, Bogorad RL, Marsico G, Nonaka H, Seifert S, Epstein-Barash H, Kuchimanchi S, Peng CG, Ruda VM *et al.*: **Rab5 is necessary for the biogenesis of the endolysosomal system in vivo**. *Nature* 2012, **485**:465-470.

51. MacDonald C, Buchkovich NJ, Stringer DK, Emr SD, Piper RC: **Cargo ubiquitination is essential for multivesicular body intralumenal vesicle formation**. *EMBO Rep* 2012, **13**:331-338.

52. Katzmann DJ, Stefan CJ, Babst M, Emr SD: **Vps27 recruits ESCRT machinery to endosomes during MVB sorting**. *J Cell Biol* 2003, **162**:413-423.

53. Raiborg C, Bache KG, Gillooly DJ, Madshus IH, Stang E, Stenmark H: **Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes**. *Nat Cell Biol* 2002, **4**:394-398.

54. Futter CE, Collinson LM, Backer JM, Hopkins CR: **Human VPS34 is required for internal vesicle formation within multivesicular endosomes**. *J Cell Biol* 2001, **155**:1251-1264.

55. • Adell MA, Vogel GF, Pakdel M, Muller M, Lindner H, Hess MW, Teis D: **Coordinated binding of Vps4 to ESCRT-III drives membrane neck constriction during MVB vesicle formation**. *J Cell Biol* 2014, **205**:33-49.
Vps4 recycles ESCRT-III subunits and also cooperates with ESCRT-III to drive distinct membrane-remodeling steps, which lead to efficient membrane scission at the end of ILV biogenesis *in vivo*.

56. Henne WM, Buchkovich NJ, Emr SD: **The ESCRT pathway**. *Dev Cell* 2011, **21**:77-91.

57. Liu XM, Sun LL, Hu W, Ding YH, Dong MQ, Du LL: **ESCRTs cooperate with a selective autophagy receptor to mediate vacuolar targeting of soluble cargos**. *Mol Cell* 2015, **59**:1035-1042.

58. Puri C, Renna M, Bento CF, Moreau K, Rubinsztein DC: **Diverse autophagosome membrane sources coalesce in recycling endosomes**. *Cell* 2013, **154**:1285-1299.

59. Edinger AL, Thompson CB: **Antigen-presenting cells control T cell proliferation by regulating amino acid availability**. *Proc Natl Acad Sci U S A* 2002, **99**:1107-1109.

60. Edinger AL, Cinalli RM, Thompson CB: **Rab7 prevents growth factor-independent survival by inhibiting cell-autonomous nutrient transporter expression**. *Dev Cell* 2003, **5**:571-582.

61. Tan X, Thapa N, Sun Y, Anderson RA: **A kinase-independent role for EGF receptor in autophagy initiation**. *Cell* 2015, **160**:145-160.

62. Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, Di Malta C, Donaudy F, Embrione V, Polishchuk RS *et al.*: **A gene network regulating lysosomal biogenesis and function**. *Science* 2009, **325**:473-477.

63. Fullgrabe J, Klionsky DJ, Joseph B: **The return of the nucleus: transcriptional and epigenetic control of autophagy**. *Nat Rev Mol Cell Biol* 2014, **15**:65-74.

64. •• Muller M, Schmidt O, Angelova M, Faserl K, Weys S, Kremser L, Pfaffenwimmer T, Dalik T, Kraft C, Trajanoski Z *et al.*: **The coordinated action of the MVB pathway and autophagy ensures cell survival during starvation**. *eLife* 2015:4.
This work shows that a catabolic cascade including the coordinated action of the MVB pathway and autophagy in yeast is essential to enter quiescence to survive nutrient limitation.

65. Kihara A, Noda T, Ishihara N, Ohsumi Y: **Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in *Saccharomyces cerevisiae***. *J Cell Biol* 2001, **152**:519-530.

66. Stack JH, Herman PK, Schu PV, Emr SD: **A membrane-associated complex containing the Vps15 protein kinase and the Vps34 PI 3-kinase is essential for protein sorting to the yeast lysosome-like vacuole**. *EMBO J* 1993, **12**:2195-2204.

67. Schu PV, Takegawa K, Fry MJ, Stack JH, Waterfield MD, Emr SD: **Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting**. *Science* 1993, **260**:88-91.

68. Odorizzi G, Babst M, Emr SD: **Fab1p PtdIns(3)P 5-kinase function essential for protein sorting in the multivesicular body**. *Cell* 1998, **95**:847-858.

69. Rusten TE, Vaccari T, Lindmo K, Rodahl LM, Nezis IP, Sem-Jacobsen C, Wendler F, Vincent JP, Brech A, Bilder D *et al.*: **ESCRTs and Fab1 regulate distinct steps of autophagy**. *Curr Biol: CB* 2007, **17**:1817-1825.

70. Ravikumar B, Imarisio S, Sarkar S, O'Kane CJ, Rubinsztein DC: **Rab5 modulates aggregation and toxicity of mutant huntingtin through macroautophagy in cell and fly models of Huntington disease**. *J Cell Sci* 2008, **121**:1649-1660.

71. Gutierrez MG, Munafo DB, Beron W, Colombo MI: **Rab7 is required for the normal progression of the autophagic pathway in mammalian cells**. *J Cell Sci* 2004, **117**:2687-2697.

72. Jager S, Bucci C, Tanida I, Ueno T, Kominami E, Saftig P, Eskelinen EL: **Role for Rab7 in maturation of late autophagic vacuoles**. *J Cell Sci* 2004, **117**:4837-4848.

73. Tooze SA, Abada A, Elazar Z: **Endocytosis and autophagy: exploitation or cooperation?** *Cold Spring Harb Perspect Biol* 2014, **6**:a018358.

74. Seglen PO, Gordon PB, Holen I, Hoyvik H: **Hepatocytic autophagy**. *Biomed Biochim Acta* 1991, **50**:373-381.

75. Filimonenko M, Stuffers S, Raiborg C, Yamamoto A, Malerod L, Fisher EM, Isaacs A, Brech A, Stenmark H, Simonsen A: **Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease**. *J Cell Biol* 2007, **179**:485-500.

76. Kaushik S, Cuervo AM: **Chaperone-mediated autophagy**. *Methods Mol Biol* 2008, **445**:227-244.

77. Massey AC, Follenzi A, Kiffin R, Zhang C, Cuervo AM: **Early cellular changes after blockage of chaperone-mediated autophagy**. *Autophagy* 2008, **4**:442-456.