Leading Edge
Review

Cell

Metabolic Control of Autophagy

Lorenzo Galluzzi,[1,2,3,4,5] Federico Pietrocola,[1,2,3,4,5,6] Beth Levine,[7,8] and Guido Kroemer[1,2,3,4,9,10,*]
[1]Equipe 11 labellisée par la Ligue Nationale contre le Cancer, Centre de Recherche des Cordeliers, 75006 Paris, France
[2]INSERM, U1138, 75006 Paris, France
[3]Université Paris Descartes, Sorbonne Paris Cité, 75005 Paris, France
[4]Université Pierre et Marie Curie, 75005 Paris, France
[5]Gustave Roussy Cancer Campus, 94805 Villejuif, France
[6]Université Paris Sud, 94805 Villejuif, France
[7]Center for Autophagy Research, Department of Internal Medicine, Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75690, USA
[8]Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[9]Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, 75015 Paris, France
[10]Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, 94805 Villejuif, France
*Correspondence: kroemer@orange.fr
http://dx.doi.org/10.1016/j.cell.2014.11.006

Macroautophagy (herein referred to as autophagy) is an evolutionarily conserved mechanism of adaptation to adverse microenvironmental conditions, including limited nutrient supplies. Several sensors interacting with the autophagic machinery have evolved to detect fluctuations in key metabolic parameters. The signal transduction cascades operating downstream of these sensors are highly interconnected to control a spatially and chronologically coordinated autophagic response that maintains the health and function of individual cells while preserving organismal homeostasis. Here, we discuss the physiological regulation of autophagy by metabolic circuitries, as well as alterations of such control in disease.

Introduction

Macroautophagy (hereafter referred to as autophagy) involves the sequestration of cytoplasmic components (which can be entire organelles, lipid vesicles, or protein aggregates) within a double-membraned vesicle, the so-called autophagosome. Autophagosomes fuse with lysosomes to generate autolysosomes, in which the autophagic cargo is degraded by acidic hydrolases. Autophagy relies on a machinery that operates in a tightly coordinated fashion and includes: (1) a multiprotein complex organized around unc-51-like autophagy activating kinase 1 (ULK1), RB1-inducible coiled-coil 1 (RB1CC1, best known as FIP200), autophagy-related 13 (ATG13), and ATG101, which triggers autophagy when the mechanistic target of rapamycin (MTOR) complex 1 (mTORC1) is inhibited; (2) a second multiprotein complex involving (among several interactors) phosphatidylinositol 3-kinase, catalytic subunit type 3 (PIK3C3, best known as vacuolar protein sorting 34, VPS34), Beclin 1 (BECN1), and autophagy/beclin-1 regulator 1 (AMBRA1), which favors the nucleation of autophagosome precursors (so-called isolation membranes or phagophores) when inhibitory signals from antiapoptotic members of the Bcl-2 protein family are blocked; (3) two transmembrane proteins, ATG9 and vacuole membrane protein 1 (VMP1), which recycle between the Golgi apparatus, endosomes, and autophagosomes, probably facilitating the recruitment of lipids to isolation membranes; (4) two ubiquitin-like (UBL) protein conjugation systems, which cooperate to catalyze the covalent attachment of ATG12 to ATG5 and ATG16-like 1 (ATG16L1) and that of phosphatidylethanolamine to microtubule-associated protein 1 light chain 3 (MAP1LC3, best known as LC3); (5) several soluble NSF attachment protein receptor (SNARE)-like proteins, which promote the fusion between autophagosomes and lysosomes; and (6) various lysosomal enzymes that hydrolyze complex carbohydrates, proteins, lipids, and nucleic acids at low pH (for review, see Mizushima [2007]).

The primary, phylogenetically conserved role of autophagy is presumably to maintain cellular homeostasis in conditions of dwindling nutrient supplies and other metabolic perturbations (e.g., hypoxia). This is achieved through the rapid mobilization of endogenous reserves, aimed at retrieving fuel for ATP synthesis as well as building blocks for essential anabolic reactions (Singh and Cuervo, 2011), coupled to a global rewiring of intracellular metabolism (Figure 1). Autophagy-deficient eukaryotic cells are more sensitive to nutrient deprivation than their wild-type counterparts (Kroemer et al., 2010), and established tumors may be addicted to autophagy as a means to cope with adverse microenvironmental conditions (Guo et al., 2013a). Moreover, mice with genetic defects in essential components of the autophagic machinery die shortly after birth partly because they fail to mobilize sufficient reserves to survive the period of starvation between placental metabolism and breast feeding (Kuma et al., 2004).

Autophagy can be relatively nonselective, targeting to lysosomal degradation virtually any portion of the cytoplasm, or it may dispose of specific subcellular compartments in a highly selective manner (Mizushima and Komatsu, 2011). Generally, autophagic responses triggered by nutrient deprivation (which mainly serve bioenergetic/metabolic functions) are of the former type, although elongated mitochondria are selectively spared from

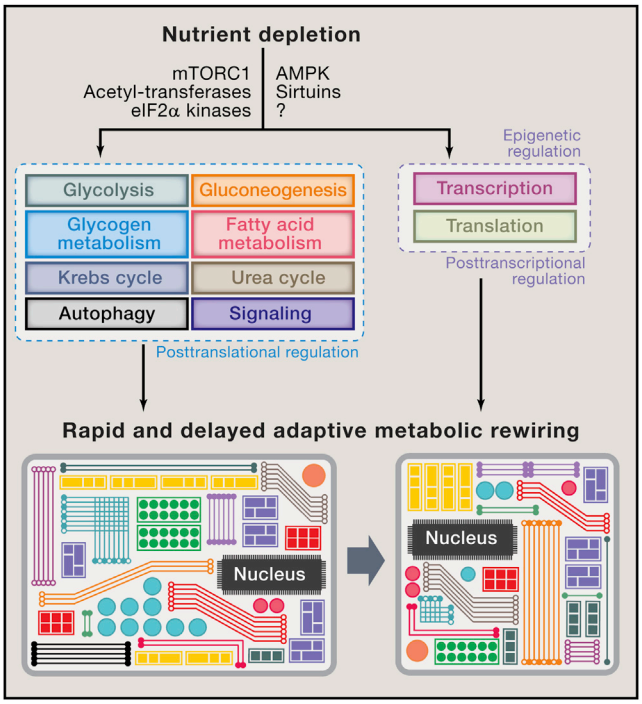

Figure 1. Cell-wide Metabolic Rewiring Associated with the Activation of Autophagy

In response to several perturbations of homeostasis, including declining levels of nutrients, cells mount an adaptive response organized around the autophagy-dependent mobilization of intracellular reserves. This response is biphasic, as it involves rapid posttranslational modifications as well as a transcriptional and translational reprogramming that has delayed consequences. Moreover, it is accompanied by a cell-wide rewiring of multiple metabolic circuitries, including both catabolic and anabolic pathways, which sustains cell survival and ensures basic cellular functions in conditions of stress. AMPK, 5′ AMP-activated protein kinase; eIF2α, eukaryotic translation initiation factor 2α; mTORC1, mechanistic target of rapamycin complex 1.

degradation in this context. Conversely, organellar damage or intracellular pathogens trigger highly selective forms of autophagy (Mizushima and Komatsu, 2011). Of note, autophagy can also actively participate in both programmed and stress-induced instances of cell death (Galluzzi et al., 2014), but this aspect will not be discussed further here.

Autophagy is crucial not only for adaptive responses to stress, but also for the maintenance of cellular homeostasis in physiological settings, at least in part because it mediates the removal of potentially dangerous constituents such as protein aggregates and dysfunctional mitochondria (Green et al., 2011). In line with this notion, the activation of autophagy at the whole-body level extends the lifespan of various model organisms, including mice (Rubinsztein et al., 2011). Moreover, defects in the autophagic machinery have been associated with numerous diseases, including aging-associated pathologies, neurodegeneration, cancer, cardiovascular disorders, and infectious/inflammatory conditions, as well as metabolic problems (Table S1 available online) (Choi et al., 2013). Thus, autophagy-incompetent mice develop both genetically and chemically driven neoplasms at a higher incidence than their autophagy-competent counterparts (Guo et al., 2013a). Various experimental models of obesity and insulin resistance are also characterized by defects in hepatic autophagy that can be efficiently targeted to prevent steatosis/steatohepatitis and improve insulin sensitivity (Yang et al., 2010). This is not surprising, given the central position occupied by the liver in the regulation of organismal metabolism and the role of autophagy in the rewiring of intracellular metabolic circuitries.

Here, we will discuss the intimate crosstalk between metabolism and autophagy, placing special emphasis on the mechanisms through which nutrients and metabolic byproducts induce or suppress autophagy at the single-cell and whole-body level, and we will explore how the metabolic regulation of autophagy influences organismal fitness in health and disease.

Metabolic Triggers of Autophagy

In isolated cells, autophagy is generally induced by limitations in ATP availability or a lack of essential nutrients, including glucose and amino acids, yet it can also be stimulated by the accumulation of specific metabolites or metabolic byproducts, such as fatty acids and ammonia (Figure 2).

Reduced Energy Charge

The metabolic status of a cell can be represented by the "energy charge" of the adenylate system (a function of intracellular ATP, ADP, and AMP concentrations), which is calculated according to the formula ([ATP] + 1/2 [ADP])/([ATP] + [ADP] + [AMP]) (Atkinson and Walton, 1967). When ATP is not actively synthesized though glycolysis or oxidative phosphorylation, the energy charge decreases in parallel with the accumulation of AMP, a condition that stimulates autophagy through protein kinase, AMP-activated (PRKA, best known as 5′ AMP-activated protein kinase, AMPK) (Hardie et al., 2012). Because AMPK utilizes ATP as a donor of phosphate groups and because several steps in the autophagic cascade consume energy, a minimum amount of ATP is required for the induction of autophagy. Thus, a rapid reduction of the energy charge below a critical limit is likely to trigger cell death rather than an adaptive autophagic response (Galluzzi et al., 2014). In cells that mostly rely on glycolysis, withdrawing glucose promotes autophagy as a result of AMP accumulation and the consequent activation of AMPK (Hardie et al., 2012). However, the inhibition of hexokinase 2 (HK2, the enzyme that catalyzes the first, rate-limiting step of glycolysis) with 2-deoxyglucose does not have the same effect because HK2 directly promotes autophagy by physically interacting and hence inhibiting mTORC1 (Roberts et al., 2014). Similarly, rotenone, a widely employed inhibitor of the respiratory chain, inhibits mitochondrial ATP synthesis but paradoxically inhibits autophagic flux (Mader et al., 2012). Thus, using toxins may not be an appropriate approach to probe complex circuitries such as those linking metabolism and autophagy. Of note, starvation, as well as hypoxia, are generally associated with increased amounts of reactive oxygen species (ROS). ROS promote autophagy by several mechanisms, including: (1) the hypoxia-inducible factor 1 (HIF-1)-dependent transactivation of BCL2/adenovirus E1B 19 kDa interacting protein 3 (*BNIP3*) and BNIP3-like (*BNIP3L*), encoding two Bcl-2 family members that potently stimulate the autophagic removal of dysfunctional mitochondria (mitophagy); (2) the ataxia-telangiectasia mutated (ATM)-dependent activation of tuberous sclerosis 2 (TSC2), a major suppressor of

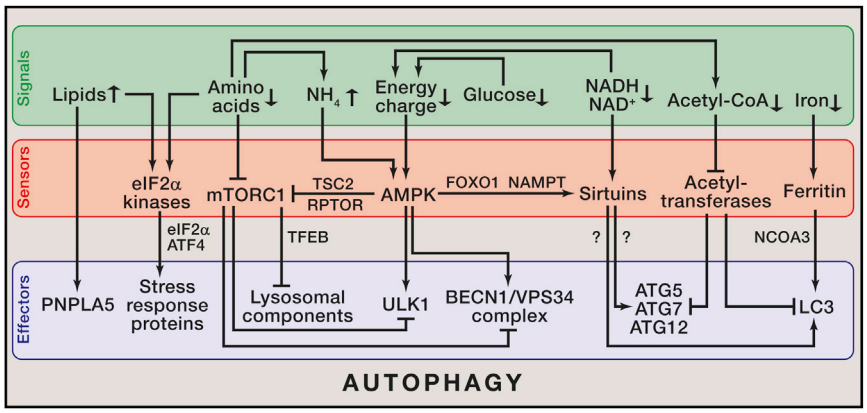

Figure 2. Metabolic Regulation of Autophagy at the Single-Cell Level

Several changes in the availability of nutrients in extracellular fluids trigger autophagy (directly or indirectly), including drops in the levels of glucose, amino acids, acetyl-CoA, and iron and decreases in the relative abundance reduced versus oxidized NAD, as well as the accumulation of specific lipids and ammonia (NH_4, a product of amino acid catabolism). Many of these metabolic cues stimulate autophagy because they inhibit mechanistic target of rapamycin complex 1 (mTORC1) or various acetyltransferases or because they activate 5′ AMP-activated protein kinase (AMPK), deacetylases of the sirtuin family, or eukaryotic translation initiation factor 2α (eIF2α) kinases. In addition, the accumulation of lipids may directly favor the formation of autophagosomes in a patatin-like phospholipase-domain-containing 5 (PNPLA5)-dependent fashion, whereas the depletion of iron has autophagy-stimulating effects upon the recognition of ferritin heavy and light chains by the autophagic adaptor nuclear receptor coactivator 4 (NCOA4). Both mTORC1 and AMPK regulate autophagy by controlling the activity of essential components of the autophagic machinery, such as unc-51 like autophagy activating kinase 1 (ULK1) or Beclin 1 (BECN1). Moreover, they are both involved in mutually regulatory interactions as well as in functional interactions with other nutrient sensors, such as sirtuins. ATF4, activating transcription factor 4; ATG, autophagy-related; FOXO1, forkhead box O1; NAMPT, nicotinamide phosphoribosyltransferase; RPTOR, regulatory-associated protein of MTOR, complex 1; TFEB, transcription factor EB; TSC2, tuberous sclerosis 2; VPS34, vacuolar protein sorting 34.

mTORC1 signaling; and (3) the oxidation-dependent activation of the essential autophagic protein ATG4 (Scherz-Shouval and Elazar, 2011). In summary, glucose deprivation and the consequent alterations in energy charge and ROS levels are potent activators of autophagy.

Reduced NADH/NAD$^+$ Ratio

In either its oxidized (NAD^+) or reduced (NADH) form, NAD is an essential substrate for multiple metabolic circuitries, including (but not limited to) glycolysis, the Krebs cycle, and oxidative phosphorylation. The exposure of cells to nutrient-free conditions causes the accumulation of NAD^+ at the expense of NADH, promoting autophagy upon activation of histone deacetylases of the sirtuin family (Houtkooper et al., 2012). Conversely, the intracellular levels of both NAD^+ and NADH fall upon the activation of NAD^+-dependent enzymes such as poly(ADP-ribose) polymerase 1 (PARP1) (Gibson and Kraus, 2012). Inhibition of these enzymes (which preserves the endogenous levels of NAD) as well as the artificial supply of NAD precursors (e.g., nicotinamide, nicotinamide riboside) potently triggers autophagy upon the activation of sirtuins, whose enzymatic activity critically relies on NAD^+ (Houtkooper et al., 2012). Thus, not only the relative abundance of NADH and NAD^+, but also the total availability of NAD has profound autophagy-modulatory effects.

Depletion of Cytosolic Acetyl-CoA

The exposure of mammalian cells to nutrient-free conditions for several hours or the overnight starvation of mice causes a significant decrease in the cytosolic levels of acetyl-CoA, which correlates with the induction of autophagy (Mariño et al., 2014). A similar effect is observed with several pharmacological or genetic interventions that inhibit (directly or by limiting substrate availability) the synthesis of acetyl-CoA within mitochondria or in the cytosol (Eisenberg et al., 2014; Mariño et al., 2014). The depletion of cytosolic acetyl-CoA stores potently stimulates autophagy, presumably because acetyl-CoA is the sole donor of acetyl groups for acetyl transferases, some of which regulate the activity of various components of the autophagic machinery at the posttranslational level (Mariño et al., 2014) or their synthesis (by acetylating histones) (Lee et al., 2014). Conversely, when intracellular acetyl-CoA levels are replenished artificially, starvation-induced autophagy is inhibited both in vitro in cultured cells and in vivo in mice (Mariño et al., 2014). Of note, the constitutive activation of *v-akt* murine thymoma viral oncogene homolog 1 (AKT1, also known as PKB), which can be triggered by oncogenic alterations such as activating mutations in Kirsten rat sarcoma viral oncogene homolog (*KRAS*), promotes acetyl-CoA synthesis upon the phosphorylation-dependent activation of ATP citrate lyase (ACLY) (Lee et al., 2014). The consequent inhibition of autophagy may contribute, at least in part, to the oncogenic effects of AKT1 hyperactivation.

Depletion of Amino Acids

Limitations in the availability of nonessential amino acids can trigger autophagy through at least four distinct, nonmutually exclusive mechanisms. First, a drop in the intracellular abundance of amino acid results in the accumulation of uncharged tRNA species. This activates eukaryotic translation initiation factor 2α kinase 4 (EIF2AK4, best known as GCN2), which blocks protein synthesis and triggers autophagy via activating transcription factor 4 (ATF4) (Ye et al., 2010). Second, the absence of amino acids in the lysosomal lumen turns off an "inside-outside" mechanism that promotes the recruitment of mTORC1 at the lysosomal surface and its activation (Zoncu et al., 2011). Third, the lack of various amino acids—in particular, leucine, glutamate, and glutamine—negatively affects intracellular acetyl-CoA stores (Mariño et al., 2014), reflecting the ability of these amino acids to efficiently feed into the Krebs cycle to generate acetyl-CoA. Fourth, the depletion of the key metabolic intermediate α-ketoglutarate caused by dwindling amino acid levels promotes autophagy along with the inhibition of proline hydroxylases (but not the stabilization of HIF-1) (Durán et al., 2013), reflecting the role of α-ketoglutarate as an obligate donor of hydroxyl groups for this class of enzymes. Proteasome inhibitors can also cause a drop in the intracellular availability of amino

acids and hence trigger autophagy. Although the relative weight of each of these pathways has not yet been determined, it appears plausible that all of these mechanisms contribute to the orchestration of optimal autophagic responses upon amino acid shortage.

Depletion of Iron

Iron is an obligate cofactor for several enzymes that catalyze redox reactions, including components of the mitochondrial respiratory chain. A fraction of cytoplasmic iron is stored within large ferritin oligomers, which can be rapidly degraded by the autophagic machinery to serve cellular needs. Drops in the intracellular availability of free iron (which can be mimicked by the administration of pharmacological chelators) activate an autophagic response that has been termed "ferritinophagy." This appears to require nuclear receptor coactivator 4 (NCOA4), which operates as an autophagic receptor for the recognition and engulfment of ferritin light and heavy chains by LC3-containing autophagosomes (Mancias et al., 2014). This example illustrates how autophagy can help cells to palliate a selective micronutrient deficiency. It will be important to explore whether similar mechanisms exist for specifically mobilizing other nutrients.

Increased Ammonia Levels

Ammonia is one of the main byproducts of the catabolism of amino acids and a potent inducer of autophagy (Eng et al., 2010). However, in contrast to the autophagic response to decreased amino acid availability, ammonia-induced autophagy does not rely on ULK1/ULK2 activation (Cheong et al., 2011) or mTORC1 inhibition (Harder et al., 2014). Rather, it seems that ammonia triggers autophagy by activating AMPK and favoring the endoplasmic reticulum (ER) stress response (Harder et al., 2014). Of note, neoplastic tissues produce high levels of ammonia as a result of an intense flux through glutaminolysis (Galluzzi et al., 2013). At least in part, this may contribute to the upregulation of the autophagic flux observed in some established neoplasms (Guo et al., 2013a).

Lipids

Both saturated and unsaturated fatty acids, such as palmitate and oleate, respectively, can stimulate autophagy, albeit through distinct mechanisms. Palmitate-induced, but not oleate-induced, autophagy requires EIF2AK2 (best known as PKR) and mitogen-activated protein kinase 8 (MAPK8, best known as JNK1) (Shen et al., 2012). Stearoyl-CoA desaturase, which converts saturated lipids into their monounsaturated counterparts, is required for starvation-induced autophagy, and the external supplementation of oleate con overcome the autophagic defect induced by stearoyl-CoA desaturase inhibitors (Ogasawara et al., 2014). Possibly, this results from the need for lipids in the generation of autophagosomes, a process that may rely on the neutral lipase patatin-like phospholipase-domain-containing 5 (PNPLA5) (Dupont et al., 2014). Enterocytes transiently store dietary lipids in triglyceride-containing droplets that localize at the ER. Such droplets trigger an immediate autophagic response that results in their capture by nascent autophagosomes and their delivery to lysosomes for degradation (Khaldoun et al., 2014). Hence, lipids can induce autophagy despite being nutrients, and this may constitute an important mechanism to avoid lipotoxicity at the cell-autonomous level.

Metabolic Sensors that Initiate Autophagy

AMPK

AMPK, one of the key energy sensors of the cell, is a heterotrimer composed of a catalytic α subunit, a scaffolding β subunit, and a regulatory γ subunit, all of which are expressed in multiple variants by mammals (α1, α2, β1, β2, γ1, γ2, and γ3). The binding of two molecules of AMP (or ADP, with lower affinity) to the γ subunit inhibits the inactivating dephosphorylation of the α subunit at T172 (Hardie et al., 2012). Hence, decreases in cellular energy charge boost the kinase activity of AMPK. The phosphorylation of AMPK α subunit at T172 can be catalyzed by calcium/calmodulin-dependent protein kinase kinase 2, β (CAMKK2) and serine/threonine kinase 11 (STK11, best known as liver kinase B1, LKB1), or stimulated (probably via an indirect mechanism) by mitogen-activated protein kinase kinase kinase 7 (MAP3K7, best known as TAK1) (Hardie et al., 2012).

TAK1 is required for the starvation-induced phosphorylation of AMPK and consequent autophagic response in cancer cells in vitro (Criollo et al., 2011), as well as in hepatocytes in vivo (Inokuchi-Shimizu et al., 2014). TAK1 activation is linked to two additional phenomena that may stimulate autophagy, namely: (1) the displacement of TAK1-binding protein 2 (TAB2) and TAB3, two TAK1 coactivators, from their autophagy-inhibitory interaction with BECN1 (Criollo et al., 2011), and (2) the activation of the IκB kinase (IKK) complex, which stimulates autophagy by phosphorylating the regulatory subunit of phosphoinositide-3-kinase (PI3K), thereby reducing its localization to cell membranes and enzymatic activity (Comb et al., 2012). Indeed, IKK is required for an optimal autophagic response to starvation in vitro (Comb et al., 2012; Criollo et al., 2011), and the ablation of the gene coding for the IKK-subunit-conserved helix-loop-helix ubiquitous kinase (CHUK, best known as IKKα), limits autophagy in pancreatic acinar cells in vivo (Li et al., 2013). Under some circumstances, AMPK can activate TAK1 (Lanna et al., 2014), suggesting that these kinases may engage in a mutually stimulatory amplification cascade. AMPK can also be activated allosterically by pharmacological agents, perhaps reflecting the existence of a hitherto unidentified (and perhaps crucial) endogenous metabolite that regulates its enzymatic activity. Among other compounds, this applies to salicylate, a phenolic phytohormone with analgesic, antipyretic, anti-inflammatory, and perhaps anticancer activity (Hawley et al., 2012). Still, it remains unclear whether the activation of AMPK is relevant to the broad pharmacological effects of aspirin, the pro-drug of salicylate.

AMPK is a master regulator of metabolism, and it stimulates autophagy by multiple mechanisms. Beyond inhibiting mTORC1 (see below), AMPK phosphorylates and activates ULK1 (Kim et al., 2011), as well as various components of the BECN1/VPS34 complex. In particular, upon glucose deprivation, AMPK phosphorylates BECN1 on S93 and S96, which augments the class III PI3K activity of VPS34, as well as VPS34 itself (on T163 and S165), which inhibits its nonautophagic functions in endosome-to-Golgi retrograde trafficking (Kim et al., 2013).

mTORC1

mTORC1 is composed of: (1) MTOR; (2) two mTORC1-specific proteins—namely, regulatory-associated protein of MTOR, complex 1 (RPTOR) and AKT1 substrate 1 (AKT1S1, best known as PRAS40); and (3) several proteins that are shared with another

MTOR-containing complex (mTORC2), i.e., DEP-domain-containing MTOR-interacting protein (DEPTOR) and MTOR-associated protein, LST8 homolog (MLST8). In response to growth factors, mTORC1 phosphorylates eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1, best known as 4-EBP1) and ribosomal protein S6 kinase (RPS6K, best known as $p70^{S6K}$), ultimately promoting protein synthesis (Shimobayashi and Hall, 2014). Activated mTORC1 suppresses autophagy by phosphorylating and inhibiting ULK1 (Kim et al., 2011), AMBRA1 and ATG14 (two autophagy-stimulatory interactors of BECN1) (Nazio et al., 2013; Yuan et al., 2013), and transcription factor EB (TFEB, see below) (Settembre et al., 2013). Of note, AMPK can inhibit mTORC1 (and hence promote autophagy) indirectly by phosphorylating and activating tuberous sclerosis 2 (a negative regulator of mTORC1), as well as directly by phosphorylating RPTOR (Hardie et al., 2012).

The availability of amino acids positively regulates mTORC1 (and hence suppresses autophagy) via multiple pathways. For instance, amino acids activate mTORC1 on the lysosomal membrane from within the lumen of the organelle, a process that involves vacuolar ATPases, a Ras-related GTP binding (RRAG)-containing complex that has been termed "Ragulator," and the mTORC1 activator Ras homolog enriched in brain (RHEB) (Sancak et al., 2010). Artificial increases in the levels of α-ketoglutarate, which can be achieved by the provision of glutamine (via glutaminolysis) or several cell-permeant precursors (i.e., dimethyl-α-ketoglutarate, trifluoromethylbenzyl-α-ketoglutarate, 5-ethyltrifluoromethylbenzyl-α-ketoglutarate), potently activate mTORC1 in cells depleted of amino acids (Mariño et al., 2014), at least in part through such a lysosomal RHEB-dependent pathway (Durán et al., 2012). Conversely, another α-ketoglutarate precursor (i.e., 1-octyl-α-ketoglutarate) appears to inhibit mTORC1, activate autophagy, and mediate lifespan-extending effects (Chin et al., 2014). The authors of this report ascribe their findings to the ability of α-ketoglutarate to inhibit mitochondrial ATP synthesis at the level of the F_1F_O-ATPase. Of note, leucine may also activate mTORC1 through the lysosomal RRAG-RHEB system. Indeed, leucine has been shown to stimulate glutaminolysis by allosterically activating glutamate dehydrogenase and to activate RRAG in the form of leucyl-tRNA synthetase (Han et al., 2012). Recently, the lysosomal RRAG-RHEB system has been suggested to contribute to the activation of mTORC1 by glucose (Efeyan et al., 2013), suggesting that AMPK may not constitute the sole sensor of glucose deprivation.

It is important to note that mTORC1 not only represses autophagy and lysosomal biogenesis, but also operates as a general regulator of anabolic reactions (Shimobayashi and Hall, 2014). Thus, similar to AMPK, mTORC1 controls several metabolic circuitries outside of the autophagic cascade, implying that chemical mTORC1 inhibitors such as rapamycin (which is approved for use in humans as an immunosuppressant to prevent the rejection of solid transplants) and other compounds commonly referred to as "rapalogs" have broad metabolic consequences that are not limited to the induction of autophagy. Moreover, mTORC1 inhibitors lose their capacity to trigger autophagy when the downstream signaling pathways are affected by oncogenic autophagy-suppressing alterations, such as the phosphorylation of BECN1 on S234 and S295 (which is catalyzed by AKT1) (Wang et al., 2012) or on Y229, Y233, and Y352, which is catalyzed by the epidermal growth factor receptor (EGFR) (Wei et al., 2013). This should be taken into consideration when mTORC1 inhibitors are employed to stimulate autophagy in cancer cells exhibiting PI3K hyperactivation or bearing activating mutations in *EGFR*.

eIF2α Kinases

The phosphorylation of eukaryotic translation initiation factor 2α (EIF2A, best known as eIF2α) on S51 is a cardinal feature of the so-called "integrated stress response," which allows cells to interrupt protein synthesis in response to the accumulation of unfolded proteins in the ER and attempt to restore homeostasis along with the activation of autophagy (Kroemer et al., 2010). The mammalian genome codes for at least four kinases that phosphorylate eIF2α—namely, EIF2AK1 (best known as HRI), EIF2AK2 (best known as PKR), EIF2AK3 (best known as PERK), and EIF2AK4 (best known as GCN2) (Silvera et al., 2010). These kinases are activated by a variety of stimuli. Limited heme availability or heavy metals like cadmium activate HRI. Double-stranded RNA or high doses of palmitate stimulate PKR. The accumulation of unfolded proteins in the ER activates PERK. Finally, the accumulation of uncharged tRNAs boosts the activity of GCN2 (Silvera et al., 2010). Of note, the response of cells expressing a nonphosphorylatable mutant of eIF2α ($EIF2A^{S51A}$) to several autophagy-inducing conditions is largely defective (Tallóczy et al., 2002). This may indicate that the efficient induction of autophagy by various stimuli requires the transactivation of multiple genes that are controlled by ATF4, which operates downstream of eIF2α (B'chir et al., 2013). Thus, it appears that the integrated stress response is closely tied to the regulation of autophagy.

Sirtuins

Sirtuins constitute a family of NAD^+-dependent class III histone deacetylases that catalyze the deacetylation of protein substrates coupled to the generation of nicotinamide and 2′-O-acetyl-ADP-ribose (Houtkooper et al., 2012). The best-studied of these enzymes, sirtuin 1 (SIRT1), is mainly located in the nucleus, where it deacetylates various histones (e.g., H1, H3, and H4) and other proteins, including transcription factors such as p53, NF-κB, forkhead box O1 (FOXO1), FOXO3, and peroxisome proliferator-activated receptor γ, coactivator 1α (PPARGC1A) (Houtkooper et al., 2012). Activation of SIRT1 with the natural polyphenol resveratrol promotes autophagy and extends the lifespan of several organisms (Lagouge et al., 2006). Moreover, overexpression of a SIRT1 mutant that exclusively localizes to the cytoplasm induces robust autophagic responses (Morselli et al., 2011), indicating that SIRT1 can trigger autophagy independently of its transcriptional functions. The cytoplasmic effectors of SIRT1-driven autophagy remain to be precisely identified, although one single report points to a direct involvement of ATG5, ATG7, ATG12 and LC3 (Lee et al., 2008).

Besides responding to increasing NAD^+ concentrations (see above), the enzymatic activity of SIRT1 changes as a function of its own expression levels. Although high-fat diet and obesity downregulate SIRT1 in several organs, both in mice and in humans, caloric restriction promotes SIRT1 expression in multiple mouse tissues (Chalkiadaki and Guarente, 2012). Because the ability of caloric restriction to increase lifespan is preserved in

Sir1$^{+/-}$ (but lost in *Sir1*$^{-/-}$) mice (Mercken et al., 2014), such an upregulation may not be involved in the lifespan-extending effects of interventions that activate SIRT1. However, results obtained with *Sir1*$^{-/-}$ mice must be taken with caution because these animals are not born at Mendelian ratios and often exhibit developmental defects (Cheng et al., 2003). Of note, AMPK may activate SIRT1 by promoting the FOXO1-dependent transactivation of the gene encoding nicotinamide phosphoribosyltransferase (NAMPT), an enzyme involved in the production of NAD^+ (Cantó et al., 2009). Thus, at least under some circumstances, SIRT1 contributes to the proautophagic activity of AMPK.

Acetyltransferases

Reductions in the intracellular pool of acetyl-CoA entail a net decrease in global protein acetylation, both in the cytoplasm (Mariño et al., 2014) and in the nucleus (Eisenberg et al., 2014). Thus, the activity of several acetyltransferases may vary as a function of the availability of acetyl-CoA, the sole donor of acetyl groups for the reactions that they catalyze. One acetyltransferase that plays a critical role in the regulation of autophagy is E1A-binding protein p300 (EP300) (Mariño et al., 2014). In cell-free systems, the activity of EP300 responds to shifts in the intracellular abundance of acetyl-CoA observed during the physiological transition from a fed to an unfed state. Moreover, the genetic or pharmacological inhibition of EP300 promotes autophagy even in conditions in which acetyl-CoA is artificially maintained at high levels (Mariño et al., 2014). EP300 reportedly acetylates and inhibits several proteins of the core autophagic machinery such as ATG5, ATG7, ATG12, and LC3 (Lee and Finkel, 2009). However, it is likely that other acetyltransferases, including members of the inhibitor of growth (ING) family, participate in the regulation of autophagy via nuclear and cytoplasmic pathways (Mariño et al., 2014). In yeast, which lacks a bona fide EP300 ortholog, several histone acetyltransferases have been implicated in the transcriptional control of autophagy (Eisenberg et al., 2009). Moreover, at least in yeast, a specific combination of deacetylation- and acetylation-mimicking mutations in histone-coding genes can cause the constitutive overexpression of core components of the autophagic machinery, resulting in increased autophagic flux and lifespan extension (Eisenberg et al., 2014).

Distinct acetyltransferases are organized in a hierarchical manner so that the inhibition of one can be coupled to the activation of another. For example, EP300 acts as a negative regulator of α tubulin acetyltransferase 1 (ATAT1, also known as MEC17), which itself is a substrate of, and can be activated by, AMPK. The activity of EP300 is also negatively regulated by AMPK-dependent phosphorylation on S89 (Yang et al., 2001). Thus, in conditions of EP300 inhibition and AMPK activation (which are intimately linked), MEC17 promotes the hyperacetylation of α-tubulin, which has autophagy-stimulatory effects (Mackeh et al., 2014). In summary, both deacetylation and hyperacetylation events contribute to autophagy, and it would be an oversimplification to state that all acetyltransferases contribute to the repression of autophagy.

Transcription Factors

The so-called ''coordinated lysosomal expression and regulation'' (CLEAR) gene network, an ensemble of genes expressed in a synchronized manner in response to perturbations of lysosomal activity (most of which are relevant for autophagy), is activated by transcription factors, including TFEB and its homolog transcription factor E3 (TFE3) (Settembre et al., 2013). TFEB is recruited to lysosomal membranes by the Ragulator, allowing for its phosphorylation at S142 and S211. Phosphorylated TFEB is sequestered by chaperones of the 14-3-3 family, which actively prevent its translocation to the nucleus. Accordingly, the substitution of TFEB S142 and S211 with alanine residues results in its constitutive translocation to the nucleus. TFEB can also be phosphorylated on S142 by mitogen-activated protein kinase 1 (MAPK1, best known as ERK2), which indeed exerts autophagy-inhibitory functions (Settembre et al., 2013). The identity of the phosphatase that dephosphorylates TFEB at these residues remains to be elucidated.

Importantly, TFEB regulates its own transcription, implying the existence of a self-amplificatory signaling loop that perpetuates the autophagic response. How such a loop is turned off remains elusive. Another transcription factor, zinc finger with KRAB and SCAN domains 3 (ZKSCAN3), functionally antagonizes TFEB. Thus, in response to starvation or mTORC1 inhibition, ZKSCAN3 translocates from the nucleus to the cytoplasm, and the knockdown of ZKSCAN3 suffices to facilitate the induction of autophagy. Indeed, ZKSCAN3 represses the transcription of more than 60 TFEB target genes involved in autophagy and lysosomal functions (Settembre et al., 2013). Whether these transcription factors truly detect metabolic perturbations or whether they simply execute autophagic responses triggered by upstream sensors such as mTORC1 remains to be determined. Irrespective of this unknown, TFEB-induced autophagy has a central role in disease protection, as the viral delivery of a TFEB-coding construct to the liver prevents the hepatic accumulation of lipid vesicles in both diet-induced and genetic models of obesity (Settembre et al., 2013). Interestingly, the nematode ortholog of TFEB (HLH-30) is required for the induction of autophagy by longevity-extending manipulations in *Caenorhabditis elegans* (Lapierre et al., 2013).

Cell-Surface Nutrient Receptors

Several G protein-coupled receptors (GPCRs) expressed on the cell surface sample the extracellular microenvironment for nutrient availability and signal to the autophagic machinery. These include (but are not limited to): (1) G protein-coupled receptor, class C, group 6, member A (GPRC6A), γ-aminobutyric acid B receptor 1 (GABBR1), calcium-sensing receptor (CASR), heterodimeric taste receptors, and various metabotropic glutamate receptors, all of which sense one or more amino acids; (2) free fatty acid receptor 1 (FFAR1) and FFAR4, which detect long-chain fatty acids; and (3) FFAR2 and FFAR3, which are activated by short-chain fatty acids (Wauson et al., 2014). The signal transduction cascades linking each of these receptors to the autophagic machinery have not yet been precisely defined, but they all presumably operate by promoting increases in the intracellular levels of inositol-1,4,5,-triphosphate and diacylglycerol, or those of cyclic AMP (Wauson et al., 2014).

In several cell types, including pancreatic β cells, cardiac myoblasts, and cervical carcinoma HeLa cells, the knockdown of either subunit of heterodimeric taste receptors—namely, taste receptor, type 1, member 1 (TAS1R1) and TAS1R3—promotes autophagy even in the presence of excess extracellular amino

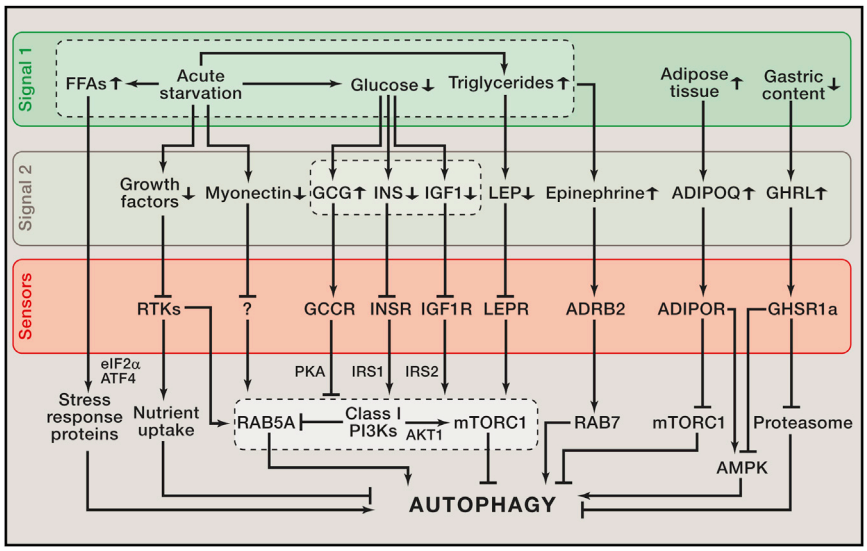

Figure 3. Metabolic Regulation of Autophagy at the Organismal Level

Acute starvation induces a stereotyped pattern of metabolic alterations, including a (limited) decrease in circulating glucose levels coupled to an increase in blood-borne triglycerides and free fatty acids (FFAs). This is generally accompanied by the secretion of glucagon (GCG), as well as by a reduction in the circulating levels of growth factors, insulin (INS), insulin-like growth factor 1 (IGF1), leptin (LEP), and myonectin. FFAs trigger autophagy as they freely enter cells and promote the inactivation of eukaryotic translation initiation factor 2α (eIF2α) coupled to the activation of activating transcription factor 4 (ATF4). Conversely, the alterations in the circulating levels of INS, IGF1, GCG, and LEP induced by starvation are sensed by specific receptors expressed at the cell surface, all of which impinge on the *v-akt* murine thymoma viral oncogene homolog 1 (AKT1)/mechanistic target of rapamycin complex 1 (mTORC1) signaling axis. In addition, drops in the availability of growth factors promote autophagy by limiting the expression of nutrient transporters. Epinephrine is also secreted in response to starvation, promoting autophagy in the periphery upon binding to adrenoceptor β2 (ADRB2). Other hormones with prominent autophagy-regulatory functions are adiponectin (ADIPOQ) and ghrelin (GHRL). The former, which is secreted by the adipose tissue, exerts pure autophagy stimulatory functions by inhibiting mTORC1 and promoting the activation of 5′ AMP-activated protein kinase (AMPK). The latter, which is secreted upon relaxation of the gastric wall, has been shown to inhibit AMPK in some circumstances, hence inhibiting autophagy, and to suppress proteasomal protein degradation in others, hence increasing autophagic flux. ADIPOR, ADIPOQ receptor; GCGR, GCG receptor; GHSR1a, growth hormone secretagogue receptor 1A; IGF1R, IGF1 receptor; INSR, INS receptor; IRS, INSR substrate; LEPR, LEP receptor; PI3K, phosphinositide-3-kinase; PKA, protein kinase A; RTK, receptor tyrosine kinase.

acids. Similarly, *Tas1r3*$^{-/-}$ mice exhibit increased autophagic responses to starvation in the heart, liver, and skeletal muscle, as compared to their wild-type counterparts (Wauson et al., 2012). Indirect evidence also suggests that the omega-3 fatty acid docosahexaenoic acid induces autophagy upon binding to FFAR4 on the cell surface (Williams-Bey et al., 2014). These results point to the possibility that GPCRs not only sense nutrients in the olfactory and gustative sensory organs, but also act in peripheral tissues to regulate autophagy in response to extracellular metabolic cues.

In summary, several systems are in place to detect fluctuations in the intracellular and/or extracellular availability of nutrients and hence initiate an autophagic response. However, it remains to be explored which among these systems preferentially respond to a global nutrient limitation (which may be caused by a reduction in blood supply) rather than to changes in the abundance of a specific molecule (which may be the result of precise metabolic perturbations). Moreover, it is not yet known whether nutrient sensors have similar activation thresholds in all cell types. It is reasonable to expect that distinct AMPK isoforms, the composition of mTORC1, the subcellular localization of acetyltransferases and sirtuins, as well as the expression pattern of GPCRs, ultimately impact on the fine regulation of autophagy.

Induction of Autophagy by Metabolic Restriction In Vivo

Although culturing cells in the absence of glucose, amino acids, or all nutrients constitutes a valid model for the induction of autophagy in vitro, such drastic alterations in the abundance of extracellular supplies do not occur in vivo, at least in mammals. Maintaining mice for 24–48 hr in the absence of food (but with free access to water) induces autophagy in close-to-all nucleated cells of the body while causing a reduction of 10%–20% in body weight (Mizushima, 2009). Yet, this does not cause a major depletion in the circulating levels of amino acids or massive, life-threatening hypoglycemia because of the autophagy-dependent mobilization of cellular stores and the systemic response to starvation involving hepatic and muscular reserves (He et al., 2012; Kuma et al., 2004). Indeed, in multicellular eukaryotes, the composition of the extracellular milieu is preserved by multiple homeostatic circuits. Moreover, the cellular availability of nutrients is not mainly dictated by their abundance but, rather, by the regulation of their uptake via specific transporters expressed on the plasma membrane (Wieman et al., 2007). Thus, the expression levels and activity of the cellular systems that ensure the uptake of various nutrients, including glucose and amino acids, are regulated by several growth factors as well as by neuroendocrine circuits (Kim and Lee, 2014) (Figure 3).

In conditions of acute starvation, the circulating levels of insulin (INS) and insulin-like growth factor 1 (IGF1) decrease while those of the insulin-like growth factor binding protein 1 (IGFBP1, an IGF1 antagonist) and glucagon (GCG) increase (Cheng et al., 2014). The consequent reduction of INS and IGF1 signaling may contribute to the inhibition of nutrient uptake and mTORC1 inactivation, favoring a compensatory autophagic response that preserves bioenergetic homeostasis (Troncoso et al., 2012). Similarly, the absence of growth factors not only limits glucose uptake upon the downregulation of plasma membrane transporters (Wieman et al., 2007), but also inhibits downstream signaling via the AKT1/mTORC1 pathway and promotes the interaction between the catalytic subunit of class I PI3Ks and the small GTPase RAB5A, hence favoring the activating interaction of the latter with VPS34 (Dou et al., 2013). A similar

suppression of AKT1/mTORC1 signaling occurs upon the activation of protein kinase A by GCG (Kondomerkos et al., 2005). Thus, drops in the extracellular availability of INS, IGF1, and growth factors coupled to increased GCG signaling provoke a robust autophagic response. Accordingly, the postnatal increase in circulating INS levels resulting from breast feeding suppresses maladaptive autophagy in cardiomyocytes, as it has been shown in mice harboring a cardiomyocyte-specific deletion of *Irs1* and *Irs2* (coding for two key transducers of INS and IGF1 signals) (Riehle et al., 2013). Moreover, the exogenous provision of IGF1 reverts some of the metabolic effects of starvation in mice (Cheng et al., 2014).

Epinephrine is secreted by adrenal glands when hypothalamic neurons detect a drop in circulating glucose levels, and the consequent activation of β-adrenergic GPCRs in peripheral tissues promotes the mobilization of triglyceride stores through a mechanism that involves autophagy (Lizaso et al., 2013). Moreover, epinephrine deficiency (owing to the ablation of the gene coding for phenylethanolamine N-methyltransferase) causes severe hepatic steatosis coupled to deficient autophagy and impaired triglyceride usage yet does not affect glucose homeostasis (Sharara-Chami et al., 2012). Intriguingly, starvation is coupled to a major increase in circulating triglycerides, and the free fatty acids resulting from their catabolism (such as oleate and palmitate) may also stimulate autophagy (Shen et al., 2012).

Additional nutrient-responsive neuroendocrine mediators, including leptin, adiponectin, ghrelin, myonectin, and others, may affect autophagic responses, establishing a complex network of autophagy-stimulatory and autophagy-inhibitory signals. Prominent autophagy inducers including starvation, physical exercise, rapamycin, resveratrol, and spermidine (a natural polyamine) cause a reduction in circulating leptin levels (He et al., 2012). In the case of exercise, this effect is lost in mice expressing a variant of B cell CLL/lymphoma 2 (BCL2) that blocks stimulus-induced (but not baseline) autophagy (He et al., 2012). Conversely, the reduction of circulating leptin caused by fasting occurs normally in *Atg7*$^{-/-}$ mice (Karsli-Uzunbas et al., 2014). The knockout of adiponectin (*ADIPOQ*), encoding a hormone secreted by the adipose tissue (and placenta), reportedly inhibits autophagy in the myocardium while aggravating diet-induced obesity and the consequent cardiac dysfunction, a series of effects that can be prevented by the administration of rapamycin (Guo et al., 2013b). Ghrelin, also known as the ''hunger hormone,'' is produced by the gastrointestinal tract when the stomach empties. In vitro, ghrelin mediates both autophagy-activating and autophagy-inhibitory functions, reflecting its ability to inhibit proteasomal protein degradation and AMPK, respectively (Bonfili et al., 2013; Wang et al., 2014). It remains to be determined which of these functions predominate in vivo in physiological versus pathological scenarios. Finally, myonectin (a skeletal muscle-derived hormone encoded by *FAM132B*) suppresses hepatic autophagy upon the stimulation of AKT1/mTORC1 signaling (Seldin et al., 2013). Taken together, these observations indicate that several neuroendocrine mediators regulate autophagy.

Interestingly, autophagy is also coupled to unconventional secretory pathways, mediating the release of a series of soluble molecules, including diazepam-binding inhibitor (DBI, best known as ACB), interleukin-1β, and interleukin-18 (Zhang and Schekman, 2013). Thus, autophagy is regulated by several neuroendocrine circuits that sense systemic nutrient availability at the same time that it affects the release of various mediators, including hormones, neurotransmitters, and cytokines. The intricacies of this regulatory network are not yet fully understood and require in-depth exploration.

Despite the limitation of in vitro studies, numerous examples suggest that energy sensors with autophagy-modulatory properties in vitro are also required for autophagy induction by caloric restriction or fasting in vivo. A ketogenic diet (i.e., a high-fat, low-carbohydrate, and low-protein diet supplemented with ketogenic essential amino acids) inhibits mTORC1 in vivo and stimulates autophagy (Xu et al., 2013). Moreover, AMPK is indispensable for myocardial adaptation to caloric restriction in mice (Chen et al., 2013), and sirtuins are required for the autophagy-dependent beneficial effects of nutrient deprivation in nonmammalian model organisms (Morselli et al., 2011) and perhaps in mice (Mercken et al., 2014).

Interestingly, mice that are starved for 24 hr exhibit a significant decrease in cytosolic acetyl-CoA in skeletal and cardiac muscles (but not in the brain) (Mariño et al., 2014), suggesting that the overall nutrient status may affect the abundance of specific intracellular metabolites. Similarly, acetyl-CoA levels drop in the livers of mice experiencing prolonged periods of caloric restriction (Hebert et al., 2013). It is not yet known whether this effect stems directly from decreased nutrient availability or rather reflects a drop in AKT1-dependent activation of ACLY as a consequence of limited INS and IGF1 signaling (Lee et al., 2014).

It appears that (some of the) nutrient sensors originally identified in vitro contribute to the regulation of autophagic responses in vivo. However, we can anticipate that future studies will unravel the major impact of nervous, endocrine, and paracrine signals in the control of autophagy at the whole-body level, contributing to the sophisticated homeostatic regulation that renders the organism adaptable to changes in the quantity and quality of nutrient supplies.

Metabolic Consequences of Autophagy

In Individual Cells

Most studies on the metabolic consequences of autophagy compare wild-type cells with cells in which genes encoding essential components of the autophagic machinery have been deleted by homologous recombination or have had their products depleted by RNA interference. Though this approach may provide reliable results in short-term experiments (performed within a few days after knockout or knockdown), it is likely to generate misleading information in long-term settings. Indeed, autophagic defects cause the accumulation of malfunctioning mitochondria and redox-active protein aggregates that, in the long term, have widespread metabolic consequences, including a reduction in mitochondrial ATP synthesis and an increased generation of genotoxic ROS.

In the presence of an intact p53 system, autophagy-deficient cells exhibit impaired proliferation in vivo (Rosenfeldt et al.,

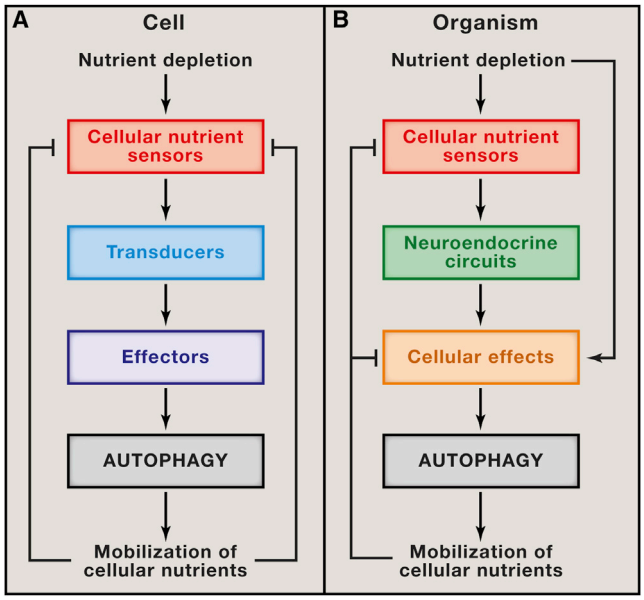

Figure 4. Regulation of Cellular and Organismal Autophagic Responses

(A) When the availability of nutrients in the extracellular fluids drops below a threshold level, sensors expressed by most (if not all) cells are activated and dispatch an autophagy-stimulating stimulus via one or more signal transducer(s). The consequent mobilization of intracellular stores restores, at least to some extent, nutrient availability, resulting in the suppression of autophagy-promoting signals.

(B) Besides mediating direct pro-autophagic effects on virtually all cells, drops in the circulating levels of several nutrients stimulate specific cell types to release neuroendocrine mediators that induce autophagy. These mediators generally trigger autophagic responses in the liver, adipose tissue, or skeletal muscle, resulting in the restoration of systemic nutrient availability and hence in the feedback inhibition of both central and cellular autophagic responses.

2013), perhaps linked to reduced glucose uptake and glycolytic flux (Lock et al., 2011). Moreover, defects in the autophagic machinery result in a marked dependency of $Braf^{V600E}$-driven lung carcinoma cells on glutamine (Strohecker et al., 2013), whereas they lower mitochondrial oxygen consumption and the levels of Krebs cycle intermediates in the context of KRAS-driven carcinogenesis (Guo et al., 2011).

Autophagy most often delays the transition between a reversible alteration of metabolic homeostasis and the generation of signals that irreversibly commit the cell to death (Galluzzi et al., 2014). Besides counteracting the depletion of energy-rich substrates, which is per se potentially lethal, autophagy limits the accumulation of permeabilized mitochondria, the organelles that regulate the intrinsic pathway of apoptosis and contribute to several instances of regulated necrosis (Galluzzi et al., 2012).

One aspect of autophagy regulation that requires further scrutiny is feedback inhibition. Indeed, it is reasonable to expect that amino acids and other energy-rich metabolites resulting from autophagy-dependent catabolic reactions act on intracellular nutrient sensors to inhibit autophagic responses (Figure 4). By reducing the autophagic flux (irrespective of the conditions that increased it), such a phenomenon may contribute to the shutdown of adaptive responses to stress that marks the recovery of homeostasis.

In Whole Organisms

One problem with the interpretation of data from mouse models bearing tissue-specific knockouts of genes encoding essential components of the autophagic machinery is that autophagy is a key process and its complete suppression invariably entails major metabolic and nonmetabolic alterations. Therefore, studies of the ablation of genes such as *Atg5* and *Atg7* in specific cell types, including subpopulations of hypothalamic neurons, pancreatic β cells, adipocytes, hepatocytes, or myocytes (Kim and Lee, 2014), may provide deeper insights into the impact of degenerative processes affecting such cells rather than the physiological contribution of autophagy to metabolic control.

Recently, the effects of the conditional deletion of *Atg7* at the whole-body level have been explored by expressing a ubiquitous transgene coding for an inducible variant of the Cre recombinase in $Atg7^{flox/flox}$ mice. In this setting, tamoxifen administration in the drinking water results in the systemic excision of both floxed *Atg7* alleles ($Atg7^{\Delta/\Delta}$ genotype) (Karsli-Uzunbas et al., 2014). Disabling autophagy in 8- to 10-week-old mice promotes an acute immunodeficiency syndrome that increases the susceptibility of mice to lethal staphylococcal infections (10% mortality 2 weeks after the administration of tamoxifen), and degenerative processes affecting all organs (notably the brain) account for the demise of all animals 6–15 weeks postknockout (Karsli-Uzunbas et al., 2014). Of note, two of the early consequences of *Atg7* deletion are the absence of liver glycogen and the replacement of white with brown adipose tissue. At this stage, i.e., 10 days after knockout, $Atg7^{\Delta/\Delta}$ mice are very sensitive to a short period (24 hr) of starvation. In particular, they exhibit profound hypoglycemia, fail to mobilize fatty acids, and succumb to starvation while manifesting massive hepatic damage (as indicated by a surge in the circulating levels of hepatic enzymes) and severe muscle wasting, as well as DNA damage responses in hepatocytes and myocytes. In this setting, glucose supplementation is sufficient to postpone death and muscle wasting, supporting the notion that $Atg7^{\Delta/\Delta}$ mice truly die from hypoglycemia in response to short periods of starvation (Karsli-Uzunbas et al., 2014). Taken together, these results suggest that the severe consequences of a complete, irreversible inhibition of the autophagic machinery prevent metabolic adaptations to fasting.

The metabolic consequences of partial autophagy inhibition have also been explored. A BCL2 variant in which three amino acids (i.e., T69, S70, and S84) have been substituted with alanine residues (referred to as $BCL2^{AAA}$) cannot be phosphorylated by JNK1. These mutations prevent the phosphorylation-dependent breakdown of BCL2/BECN1 complexes, hence allowing for baseline, but not stimulus-induced, autophagy (He et al., 2012). Thus, mice expressing $BCL2^{AAA}$ at the whole-body level display lower degrees of exercise-induced autophagy in skeletal muscles than their wild-type counterparts. This correlates with reduced physical endurance and a decreased sensitivity of skeletal muscles to exercise-induced insulin. Moreover, although a high-fat diet causes obesity and type 2 diabetes in both control and $BCL2^{AAA}$-expressing mice, the beneficial effects of exercise training on diabetes are lost in the latter. Very similar results were obtained in $Becn1^{+/-}$ mice (He et al., 2012). These findings suggest that autophagy in skeletal muscles may contribute to the systemic beneficial effects of exercise.

Becn1$^{+/-}$ mice of 16–24 months of age also exhibit increased lipid accumulation in the liver as compared to their age-matched wild-type counterparts (Amir and Czaja, 2011). Moreover, in a mosaic *Atg5* knockout model (in which only a fraction of tissues, including the liver, exhibit autophagic defects), hepatocytes became highly loaded with lipid droplets by 19 months of age (Takamura et al., 2011), supporting the idea that autophagy may counteract steatosis in a cell-autonomous fashion.

The overexpression of essential components of the autophagic machinery may yield useful information regarding the contribution of autophagy to whole-body physiology, provided that such a manipulation is not deleterious for specific cell types. For example, the transgenic expression of *Atg5* under the control of a universal, moderate promoter extends the lifespan of mice by 17.2% (Pyo et al., 2013). This suggests that promoting autophagy to some extent is nontoxic. Importantly, Atg5-overexpressing mice not only exhibit a lean phenotype, reduced hepatic levels of triglycerides, increased glucose clearance, and insulin sensitivity as compared to their wild-type counterparts, but are also protected against diet-induced obesity and insulin resistance (Pyo et al., 2013). These findings support a general anti-obesity and anti-diabetes role of autophagy.

Nutritional interventions and other (pharmacological or genetic) inducers of autophagy may also be used to manipulate metabolism in vivo. Ample evidence indicates that caloric restriction or specific nutritional manipulations (e.g., methionine restriction, polyamine supplementation) can increase the longevity of nonmammalian organisms (such as yeast, nematodes, and flies) in an autophagy-dependent manner. Similarly, rapamycin and several other experimental inducers of autophagy (such as resveratrol and spermidine) extend the lifespan of nonmammalian species in an autophagy-dependent fashion (Rubinsztein et al., 2011). Rapamycin also increases the longevity of laboratory mice and reverses age-related cardiac dysfunction, even when administered late in life (Harrison et al., 2009). However, it has not yet been determined whether autophagy is required for the lifespan-extending effects of rapamycin on mice. Moreover, rapamycin may promote type 2 diabetes, probably due to inhibition of mTORC2 (Lamming et al., 2012).

Future studies must determine which, if any, dosing schedule might reduce the immunosuppressive and pro-diabetic side effects of rapamycin or whether rapamycin should be replaced by more specific mTORC1 inhibitors (Li et al., 2014), other drugs that mimic the effects of caloric restriction (i.e., that reduce acetyl-CoA levels, such as ACLY inhibitors; stimulate sirtuins, such as nicotinamide or resveratrol; and/or inhibit acetyltransferases, such as spermidine) (Madeo et al., 2014), or agents that specifically activate the autophagic machinery (such as a cell-permeable peptide that derepresses BECN1) (Shoji-Kawata et al., 2013). Indeed, although accumulating evidence indicates that nicotinamide and resveratrol have profound anti-obesity and anti-diabetes effects (Cantó et al., 2012; Lagouge et al., 2006), it remains elusive to what extent such activity is mediated by autophagy.

Both rapamycin and nicotinamide riboside can be used to treat experimental mitochondriopathies. In particular, rapamycin improves the clinical course of mice lacking a mitochondrial respiratory chain subunit (NADH dehydrogenase [ubiquinone] Fe-S protein 4, 18 kDa, Ndufs4), which develop brain lesions similar to those associated with the Leigh syndrome (Johnson et al., 2013). Similarly, nicotinamide riboside causes a significant improvement in the symptoms of mice carrying a dominant mutation in the gene coding for progressive external ophthalmoplegia 1 (Peo1, a mitochondrial replicative helicase), which results in progressive mitochondrial myopathy upon the accumulation of mutated mtDNA (Khan et al., 2014). Moreover, nicotinamide riboside improves the mitochondrial defects and intolerance to physical exercise of mice expressing a pathologic variant of SCO cytochrome oxidase-deficient homolog 2 (Sco2) in the absence of endogenous *Sco2* alleles, which develop a mitochondrial disease model characterized by impaired biogenesis of cytochrome *c* oxidase (Cerutti et al., 2014). Because autophagy plays a major role in mitochondrial quality control, a chronic increase in autophagy could contribute to the beneficial effects of these compounds on diseases with limited therapeutic options. This possibility remains to be addressed.

In summary, we are currently witnessing the development of new pharmacological and genetic methods to manipulate (induce or suppress) autophagy, which should facilitate the exploration of this key pathway in physiological scenarios. It will be particularly interesting to examine autophagy by techniques that permit its partial and transient modulation, either in the entire organism or in defined organs. Beyond mechanistic insights, such an approach will yield information on the therapeutic utility and potential long-term side effects of autophagy-modulating measures.

Conclusions and Perspectives

Undoubtedly, metabolism regulates autophagy, and autophagy has a profound impact on metabolism. As a major manifestation of this tight interrelationship, the autophagy-dependent mobilization of cellular and organismal reserves triggers negative feedback circuitries that inhibit autophagy at both the single-cell and systemic level (Figure 4). Autophagy is a complex process that requires a major degree of coordination among distinct molecular systems, ensuring that the initial sequestration of the autophagic cargo in autophagosomes leads to lysosomal degradation. Indeed, the induction of autophagy by heterogeneous interventions provokes a relatively homogeneous response characterized by the activation of specific kinases (AMPK, IKK, JNK1, TAK1, ULK1, VPS34), the inhibition of others (such as mTORC1), protein deacetylation reactions (at least in part ensuring the activation of SIRT1 and/or the inhibition of EP300), and the reversal of inhibitory interactions such as those between BECN1 and Bcl-2 family members.

Such a tight coordination may be achieved by several mechanisms, including (but not limited to): (1) mutually stimulatory pro-autophagic interactions among nutrient sensors (e.g., AMPK and SIRT1, AMPK and mTORC1), (2) the direct activation of several pro-autophagic factors by nutrient sensors (e.g., ULK1 and TFEB, which are regulated by mTORC1), (3) positive interactions among essential molecules involved in distinct steps of the autophagic process (e.g., the phosphorylation of BECN1 and AMBRA1 by ULK1; the phosphorylation of AMBRA1 by

mTORC1); and (4) synchronized rearrangements of key factors in functionally distinct multiprotein complexes. This may explain why distinct primary signals can stimulate stereotyped changes in several supramolecular complexes involved in the regulation of autophagy.

An emerging theme is that autophagy responds to the depletion of a panel of nutrients by mobilizing intracellular reserves. Still, it is not clear yet whether distinct types of nutrient deficiencies may cause a highly specific and graduated autophagic response resembling the one triggered by iron deficiency. Thus, one might speculate that fluctuations in the abundance of specific nutrients might stimulate an autophagic response (in terms of autophagic cargo and cell types involved) that differs from the one induced by indiscriminate caloric restriction. Irrespective of these and other unknowns, autophagy exerts major homeostatic control on both cellular and organismal metabolism. Thus, we anticipate that pathological alterations of autophagy and their therapeutic correction will occupy a central stage in future clinical practice.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2014.11.006.

ACKNOWLEDGMENTS

We are indebted to Erika Vacchelli (Centre de Recherche des Cordeliers) for help with table preparation. G.K. is supported by the Ligue contre le Cancer (équipe labelisée); Agence National de la Recherche (ANR); Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; Institut National du Cancer (INCa); Fondation Bettencourt-Schueller; Fondation de France; Fondation pour la Recherche Médicale (FRM); the European Commission (ArtForce); the European Research Council (ERC); the E-Rare-2 project, the LabEx Immuno-Oncology; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); the SIRIC Cancer Research and Personalized Medicine (CARPEM); and the Paris Alliance of Cancer Research Institutes (PACRI). B.L. is supported by NIH grants RO1 CA109618 and U19 AI109725 and a Cancer Prevention Research Institute of Texas grant RP120718-PI.

REFERENCES

Amir, M., and Czaja, M.J. (2011). Autophagy in nonalcoholic steatohepatitis. Expert Rev. Gastroenterol. Hepatol. *5*, 159–166.

Atkinson, D.E., and Walton, G.M. (1967). Adenosine triphosphate conservation in metabolic regulation. Rat liver citrate cleavage enzyme. J. Biol. Chem. *242*, 3239–3241.

B'chir, W., Maurin, A.C., Carraro, V., Averous, J., Jousse, C., Muranishi, Y., Parry, L., Stepien, G., Fafournoux, P., and Bruhat, A. (2013). The eIF2α/ATF4 pathway is essential for stress-induced autophagy gene expression. Nucleic Acids Res. *41*, 7683–7699.

Bonfili, L., Cuccioloni, M., Cecarini, V., Mozzicafreddo, M., Palermo, F.A., Cocci, P., Angeletti, M., and Eleuteri, A.M. (2013). Ghrelin induces apoptosis in colon adenocarcinoma cells via proteasome inhibition and autophagy induction. Apoptosis *18*, 1188–1200.

Cantó, C., Gerhart-Hines, Z., Feige, J.N., Lagouge, M., Noriega, L., Milne, J.C., Elliott, P.J., Puigserver, P., and Auwerx, J. (2009). AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature *458*, 1056–1060.

Cantó, C., Houtkooper, R.H., Pirinen, E., Youn, D.Y., Oosterveer, M.H., Cen, Y., Fernandez-Marcos, P.J., Yamamoto, H., Andreux, P.A., Cettour-Rose, P., et al. (2012). The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab. *15*, 838–847.

Cerutti, R., Pirinen, E., Lamperti, C., Marchet, S., Sauve, A.A., Li, W., Leoni, V., Schon, E.A., Dantzer, F., Auwerx, J., et al. (2014). NAD(+)-dependent activation of Sirt1 corrects the phenotype in a mouse model of mitochondrial disease. Cell Metab. *19*, 1042–1049.

Chalkiadaki, A., and Guarente, L. (2012). Sirtuins mediate mammalian metabolic responses to nutrient availability. Nat. Rev. Endocrinol. *8*, 287–296.

Chen, K., Kobayashi, S., Xu, X., Viollet, B., and Liang, Q. (2013). AMP activated protein kinase is indispensable for myocardial adaptation to caloric restriction in mice. PLoS ONE *8*, e59682.

Cheng, H.L., Mostoslavsky, R., Saito, S., Manis, J.P., Gu, Y., Patel, P., Bronson, R., Appella, E., Alt, F.W., and Chua, K.F. (2003). Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. Proc. Natl. Acad. Sci. USA *100*, 10794–10799.

Cheng, C.W., Adams, G.B., Perin, L., Wei, M., Zhou, X., Lam, B.S., Da Sacco, S., Mirisola, M., Quinn, D.I., Dorff, T.B., et al. (2014). Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell Stem Cell *14*, 810–823.

Cheong, H., Lindsten, T., Wu, J., Lu, C., and Thompson, C.B. (2011). Ammonia-induced autophagy is independent of ULK1/ULK2 kinases. Proc. Natl. Acad. Sci. USA *108*, 11121–11126.

Chin, R.M., Fu, X., Pai, M.Y., Vergnes, L., Hwang, H., Deng, G., Diep, S., Lomenick, B., Meli, V.S., Monsalve, G.C., et al. (2014). The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. Nature *510*, 397–401.

Choi, A.M., Ryter, S.W., and Levine, B. (2013). Autophagy in human health and disease. N. Engl. J. Med. *368*, 651–662.

Comb, W.C., Hutti, J.E., Cogswell, P., Cantley, L.C., and Baldwin, A.S. (2012). p85α SH2 domain phosphorylation by IKK promotes feedback inhibition of PI3K and Akt in response to cellular starvation. Mol. Cell *45*, 719–730.

Criollo, A., Niso-Santano, M., Malik, S.A., Michaud, M., Morselli, E., Mariño, G., Lachkar, S., Arkhipenko, A.V., Harper, F., Pierron, G., et al. (2011). Inhibition of autophagy by TAB2 and TAB3. EMBO J. *30*, 4908–4920.

Dou, Z., Pan, J.A., Dbouk, H.A., Ballou, L.M., DeLeon, J.L., Fan, Y., Chen, J.S., Liang, Z., Li, G., Backer, J.M., et al. (2013). Class IA PI3K p110β subunit promotes autophagy through Rab5 small GTPase in response to growth factor limitation. Mol. Cell *50*, 29–42.

Dupont, N., Chauhan, S., Arko-Mensah, J., Castillo, E.F., Masedunskas, A., Weigert, R., Robenek, H., Proikas-Cezanne, T., and Deretic, V. (2014). Neutral lipid stores and lipase PNPLA5 contribute to autophagosome biogenesis. Curr. Biol. *24*, 609–620.

Durán, R.V., Oppliger, W., Robitaille, A.M., Heiserich, L., Skendaj, R., Gottlieb, E., and Hall, M.N. (2012). Glutaminolysis activates Rag-mTORC1 signaling. Mol. Cell *47*, 349–358.

Durán, R.V., MacKenzie, E.D., Boulahbel, H., Frezza, C., Heiserich, L., Tardito, S., Bussolati, O., Rocha, S., Hall, M.N., and Gottlieb, E. (2013). HIF-independent role of prolyl hydroxylases in the cellular response to amino acids. Oncogene *32*, 4549–4556.

Efeyan, A., Zoncu, R., Chang, S., Gumper, I., Snitkin, H., Wolfson, R.L., Kirak, O., Sabatini, D.D., and Sabatini, D.M. (2013). Regulation of mTORC1 by the Rag GTPases is necessary for neonatal autophagy and survival. Nature *493*, 679–683.

Eisenberg, T., Knauer, H., Schauer, A., Büttner, S., Ruckenstuhl, C., Carmona-Gutierrez, D., Ring, J., Schroeder, S., Magnes, C., Antonacci, L., et al. (2009). Induction of autophagy by spermidine promotes longevity. Nat. Cell Biol. *11*, 1305–1314.

Eisenberg, T., Schroeder, S., Andryushkova, A., Pendl, T., Küttner, V., Bhukel, A., Mariño, G., Pietrocola, F., Harger, A., Zimmermann, A., et al. (2014). Nucleocytosolic depletion of the energy metabolite acetyl-coenzyme a stimulates autophagy and prolongs lifespan. Cell Metab. *19*, 431–444.

Eng, C.H., Yu, K., Lucas, J., White, E., and Abraham, R.T. (2010). Ammonia derived from glutaminolysis is a diffusible regulator of autophagy. Sci. Signal. *3*, ra31.

Galluzzi, L., Kepp, O., and Kroemer, G. (2012). Mitochondria: master regulators of danger signalling. Nat. Rev. Mol. Cell Biol. *13*, 780–788.

Galluzzi, L., Kepp, O., Vander Heiden, M.G., and Kroemer, G. (2013). Metabolic targets for cancer therapy. Nat. Rev. Drug Discov. *12*, 829–846.

Galluzzi, L., Bravo-San Pedro, J.M., Vitale, I., Aaronson, S.A., Abrams, J.M., Adam, D., Alnemri, E.S., Altucci, L., Andrews, D., Annicchiarico-Petruzzelli, M., et al. (2014). Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. Cell Death Differ., in press.

Gibson, B.A., and Kraus, W.L. (2012). New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. Nat. Rev. Mol. Cell Biol. *13*, 411–424.

Green, D.R., Galluzzi, L., and Kroemer, G. (2011). Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. Science *333*, 1109–1112.

Guo, J.Y., Chen, H.Y., Mathew, R., Fan, J., Strohecker, A.M., Karsli-Uzunbas, G., Kamphorst, J.J., Chen, G., Lemons, J.M., Karantza, V., et al. (2011). Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev. *25*, 460–470.

Guo, J.Y., Xia, B., and White, E. (2013a). Autophagy-mediated tumor promotion. Cell *155*, 1216–1219.

Guo, R., Zhang, Y., Turdi, S., and Ren, J. (2013b). Adiponectin knockout accentuates high fat diet-induced obesity and cardiac dysfunction: role of autophagy. Biochim. Biophys. Acta *1832*, 1136–1148.

Han, J.M., Jeong, S.J., Park, M.C., Kim, G., Kwon, N.H., Kim, H.K., Ha, S.H., Ryu, S.H., and Kim, S. (2012). Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. Cell *149*, 410–424.

Harder, L.M., Bunkenborg, J., and Andersen, J.S. (2014). Inducing autophagy: a comparative phosphoproteomic study of the cellular response to ammonia and rapamycin. Autophagy *10*, 339–355.

Hardie, D.G., Ross, F.A., and Hawley, S.A. (2012). AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat. Rev. Mol. Cell Biol. *13*, 251–262.

Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., Nadon, N.L., Wilkinson, J.E., Frenkel, K., Carter, C.S., et al. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature *460*, 392–395.

Hawley, S.A., Fullerton, M.D., Ross, F.A., Schertzer, J.D., Chevtzoff, C., Walker, K.J., Peggie, M.W., Zibrova, D., Green, K.A., Mustard, K.J., et al. (2012). The ancient drug salicylate directly activates AMP-activated protein kinase. Science *336*, 918–922.

He, C., Bassik, M.C., Moresi, V., Sun, K., Wei, Y., Zou, Z., An, Z., Loh, J., Fisher, J., Sun, Q., et al. (2012). Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. Nature *481*, 511–515.

Hebert, A.S., Dittenhafer-Reed, K.E., Yu, W., Bailey, D.J., Selen, E.S., Boersma, M.D., Carson, J.J., Tonelli, M., Balloon, A.J., Higbee, A.J., et al. (2013). Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. Mol. Cell *49*, 186–199.

Houtkooper, R.H., Pirinen, E., and Auwerx, J. (2012). Sirtuins as regulators of metabolism and healthspan. Nat. Rev. Mol. Cell Biol. *13*, 225–238.

Inokuchi-Shimizu, S., Park, E.J., Roh, Y.S., Yang, L., Zhang, B., Song, J., Liang, S., Pimienta, M., Taniguchi, K., Wu, X., et al. (2014). TAK1-mediated autophagy and fatty acid oxidation prevent hepatosteatosis and tumorigenesis. J. Clin. Invest. *124*, 3566–3578.

Johnson, S.C., Yanos, M.E., Kayser, E.B., Quintana, A., Sangesland, M., Castanza, A., Uhde, L., Hui, J., Wall, V.Z., Gagnidze, A., et al. (2013). mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome. Science *342*, 1524–1528.

Karsli-Uzunbas, G., Guo, J.Y., Price, S., Teng, X., Laddha, S.V., Khor, S., Kalaany, N.Y., Jacks, T., Chan, C.S., Rabinowitz, J.D., and White, E. (2014). Autophagy is required for glucose homeostasis and lung tumor maintenance. Cancer Discov. *4*, 914–927.

Khaldoun, S.A., Emond-Boisjoly, M.A., Chateau, D., Carrière, V., Lacasa, M., Rousset, M., Demignot, S., and Morel, E. (2014). Autophagosomes contribute to intracellular lipid distribution in enterocytes. Mol. Biol. Cell *25*, 118–132.

Khan, N.A., Auranen, M., Paetau, I., Pirinen, E., Euro, L., Forsström, S., Pasila, L., Velagapudi, V., Carroll, C.J., Auwerx, J., and Suomalainen, A. (2014). Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3. EMBO Mol. Med. *6*, 721–731.

Kim, K.H., and Lee, M.S. (2014). Autophagy—a key player in cellular and body metabolism. Nat. Rev. Endocrinol. *10*, 322–337.

Kim, J., Kundu, M., Viollet, B., and Guan, K.L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat. Cell Biol. *13*, 132–141.

Kim, J., Kim, Y.C., Fang, C., Russell, R.C., Kim, J.H., Fan, W., Liu, R., Zhong, Q., and Guan, K.L. (2013). Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. Cell *152*, 290–303.

Kondomerkos, D.J., Kalamidas, S.A., Kotoulas, O.B., and Hann, A.C. (2005). Glycogen autophagy in the liver and heart of newborn rats. The effects of glucagon, adrenalin or rapamycin. Histol. Histopathol. *20*, 689–696.

Kroemer, G., Mariño, G., and Levine, B. (2010). Autophagy and the integrated stress response. Mol. Cell *40*, 280–293.

Kuma, A., Hatano, M., Matsui, M., Yamamoto, A., Nakaya, H., Yoshimori, T., Ohsumi, Y., Tokuhisa, T., and Mizushima, N. (2004). The role of autophagy during the early neonatal starvation period. Nature *432*, 1032–1036.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., et al. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell *127*, 1109–1122.

Lamming, D.W., Ye, L., Katajisto, P., Goncalves, M.D., Saitoh, M., Stevens, D.M., Davis, J.G., Salmon, A.B., Richardson, A., Ahima, R.S., et al. (2012). Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science *335*, 1638–1643.

Lanna, A., Henson, S.M., Escors, D., and Akbar, A.N. (2014). The kinase p38 activated by the metabolic regulator AMPK and scaffold TAB1 drives the senescence of human T cells. Nat. Immunol. *15*, 965–972.

Lapierre, L.R., De Magalhaes Filho, C.D., McQuary, P.R., Chu, C.C., Visvikis, O., Chang, J.T., Gelino, S., Ong, B., Davis, A.E., et al. (2013). The TFEB orthologue HLH-30 regulates autophagy and modulates longevity in Caenorhabditis elegans. Nat. Commun. *4*, 2267.

Lee, I.H., and Finkel, T. (2009). Regulation of autophagy by the p300 acetyltransferase. J. Biol. Chem. *284*, 6322–6328.

Lee, I.H., Cao, L., Mostoslavsky, R., Lombard, D.B., Liu, J., Bruns, N.E., Tsokos, M., Alt, F.W., and Finkel, T. (2008). A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. Proc. Natl. Acad. Sci. USA *105*, 3374–3379.

Lee, J.V., Carrer, A., Shah, S., Snyder, N.W., Wei, S., Venneti, S., Worth, A.J., Yuan, Z.F., Lim, H.W., Liu, S., et al. (2014). Akt-dependent metabolic reprogramming regulates tumor cell histone acetylation. Cell Metab. *20*, 306–319.

Li, N., Wu, X., Holzer, R.G., Lee, J.H., Todoric, J., Park, E.J., Ogata, H., Gukovskaya, A.S., Gukovsky, I., Pizzo, D.P., et al. (2013). Loss of acinar cell IKKα triggers spontaneous pancreatitis in mice. J. Clin. Invest. *123*, 2231–2243.

Li, J., Kim, S.G., and Blenis, J. (2014). Rapamycin: one drug, many effects. Cell Metab. *19*, 373–379.

Lizaso, A., Tan, K.T., and Lee, Y.H. (2013). β-adrenergic receptor-stimulated lipolysis requires the RAB7-mediated autolysosomal lipid degradation. Autophagy *9*, 1228–1243.

Lock, R., Roy, S., Kenific, C.M., Su, J.S., Salas, E., Ronen, S.M., and Debnath, J. (2011). Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. Mol. Biol. Cell *22*, 165–178.

Mackeh, R., Lorin, S., Ratier, A., Mejdoubi-Charef, N., Baillet, A., Bruneel, A., Hamaï, A., Codogno, P., Poüs, C., and Perdiz, D. (2014). Reactive oxygen species, AMP-activated protein kinase, and the transcription cofactor p300 regulate α-tubulin acetyltransferase-1 (αTAT-1/MEC-17)-dependent microtubule hyperacetylation during cell stress. J. Biol. Chem. *289*, 11816–11828.

Madeo, F., Pietrocola, F., Eisenberg, T., and Kroemer, G. (2014). Caloric restriction mimetics: towards a molecular definition. Nat. Rev. Drug Discov. *13*, 727–740.

Mader, B.J., Pivtoraiko, V.N., Flippo, H.M., Klocke, B.J., Roth, K.A., Mangieri, L.R., and Shacka, J.J. (2012). Rotenone inhibits autophagic flux prior to inducing cell death. ACS Chem. Neurosci. *3*, 1063–1072.

Mancias, J.D., Wang, X., Gygi, S.P., Harper, J.W., and Kimmelman, A.C. (2014). Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. Nature *509*, 105–109.

Mariño, G., Pietrocola, F., Eisenberg, T., Kong, Y., Malik, S.A., Andryushkova, A., Schroeder, S., Pendl, T., Harger, A., Niso-Santano, M., et al. (2014). Regulation of autophagy by cytosolic acetyl-coenzyme A. Mol. Cell *53*, 710–725.

Mercken, E.M., Hu, J., Krzysik-Walker, S., Wei, M., Li, Y., McBurney, M.W., de Cabo, R., and Longo, V.D. (2014). SIRT1 but not its increased expression is essential for lifespan extension in caloric-restricted mice. Aging Cell *13*, 193–196.

Mizushima, N. (2007). Autophagy: process and function. Genes Dev. *21*, 2861–2873.

Mizushima, N. (2009). Methods for monitoring autophagy using GFP-LC3 transgenic mice. Methods Enzymol. *452*, 13–23.

Mizushima, N., and Komatsu, M. (2011). Autophagy: renovation of cells and tissues. Cell *147*, 728–741.

Morselli, E., Mariño, G., Bennetzen, M.V., Eisenberg, T., Megalou, E., Schroeder, S., Cabrera, S., Bénit, P., Rustin, P., Criollo, A., et al. (2011). Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. J. Cell Biol. *192*, 615–629.

Nazio, F., Strappazzon, F., Antonioli, M., Bielli, P., Cianfanelli, V., Bordi, M., Gretzmeier, C., Dengjel, J., Piacentini, M., Fimia, G.M., and Cecconi, F. (2013). mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. Nat. Cell Biol. *15*, 406–416.

Ogasawara, Y., Itakura, E., Kono, N., Mizushima, N., Arai, H., Nara, A., Mizukami, T., and Yamamoto, A. (2014). Stearoyl-CoA desaturase 1 activity is required for autophagosome formation. J. Biol. Chem. *289*, 23938–23950.

Pyo, J.O., Yoo, S.M., Ahn, H.H., Nah, J., Hong, S.H., Kam, T.I., Jung, S., and Jung, Y.K. (2013). Overexpression of Atg5 in mice activates autophagy and extends lifespan. Nat. Commun. *4*, 2300.

Riehle, C., Wende, A.R., Sena, S., Pires, K.M., Pereira, R.O., Zhu, Y., Bugger, H., Frank, D., Bevins, J., Chen, D., et al. (2013). Insulin receptor substrate signaling suppresses neonatal autophagy in the heart. J. Clin. Invest. *123*, 5319–5333.

Roberts, D.J., Tan-Sah, V.P., Ding, E.Y., Smith, J.M., and Miyamoto, S. (2014). Hexokinase-II positively regulates glucose starvation-induced autophagy through TORC1 inhibition. Mol. Cell *53*, 521–533.

Rosenfeldt, M.T., O'Prey, J., Morton, J.P., Nixon, C., MacKay, G., Mrowinska, A., Au, A., Rai, T.S., Zheng, L., Ridgway, R., et al. (2013). p53 status determines the role of autophagy in pancreatic tumour development. Nature *504*, 296–300.

Rubinsztein, D.C., Mariño, G., and Kroemer, G. (2011). Autophagy and aging. Cell *146*, 682–695.

Sancak, Y., Bar-Peled, L., Zoncu, R., Markhard, A.L., Nada, S., and Sabatini, D.M. (2010). Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell *141*, 290–303.

Scherz-Shouval, R., and Elazar, Z. (2011). Regulation of autophagy by ROS: physiology and pathology. Trends Biochem. Sci. *36*, 30–38.

Seldin, M.M., Lei, X., Tan, S.Y., Stanson, K.P., Wei, Z., and Wong, G.W. (2013). Skeletal muscle-derived myonectin activates the mammalian target of rapamycin (mTOR) pathway to suppress autophagy in liver. J. Biol. Chem. *288*, 36073–36082.

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

Sharara-Chami, R.I., Zhou, Y., Ebert, S., Pacak, K., Ozcan, U., and Majzoub, J.A. (2012). Epinephrine deficiency results in intact glucose counter-regulation, severe hepatic steatosis and possible defective autophagy in fasting mice. Int. J. Biochem. Cell Biol. *44*, 905–913.

Shen, S., Niso-Santano, M., Adjemian, S., Takehara, T., Malik, S.A., Minoux, H., Souquere, S., Mariño, G., Lachkar, S., Senovilla, L., et al. (2012). Cytoplasmic STAT3 represses autophagy by inhibiting PKR activity. Mol. Cell *48*, 667–680.

Shimobayashi, M., and Hall, M.N. (2014). Making new contacts: the mTOR network in metabolism and signalling crosstalk. Nat. Rev. Mol. Cell Biol. *15*, 155–162.

Shoji-Kawata, S., Sumpter, R., Leveno, M., Campbell, G.R., Zou, Z., Kinch, L., Wilkins, A.D., Sun, Q., Pallauf, K., MacDuff, D., et al. (2013). Identification of a candidate therapeutic autophagy-inducing peptide. Nature *494*, 201–206.

Silvera, D., Formenti, S.C., and Schneider, R.J. (2010). Translational control in cancer. Nat. Rev. Cancer *10*, 254–266.

Singh, R., and Cuervo, A.M. (2011). Autophagy in the cellular energetic balance. Cell Metab. *13*, 495–504.

Strohecker, A.M., Guo, J.Y., Karsli-Uzunbas, G., Price, S.M., Chen, G.J., Mathew, R., McMahon, M., and White, E. (2013). Autophagy sustains mitochondrial glutamine metabolism and growth of BrafV600E-driven lung tumors. Cancer Discov. *3*, 1272–1285.

Takamura, A., Komatsu, M., Hara, T., Sakamoto, A., Kishi, C., Waguri, S., Eishi, Y., Hino, O., Tanaka, K., and Mizushima, N. (2011). Autophagy-deficient mice develop multiple liver tumors. Genes Dev. *25*, 795–800.

Tallóczy, Z., Jiang, W., Virgin, H.W., 4th, Leib, D.A., Scheuner, D., Kaufman, R.J., Eskelinen, E.L., and Levine, B. (2002). Regulation of starvation- and virus-induced autophagy by the eIF2alpha kinase signaling pathway. Proc. Natl. Acad. Sci. USA *99*, 190–195.

Troncoso, R., Vicencio, J.M., Parra, V., Nemchenko, A., Kawashima, Y., Del Campo, A., Toro, B., Battiprolu, P.K., Aranguiz, P., Chiong, M., et al. (2012). Energy-preserving effects of IGF-1 antagonize starvation-induced cardiac autophagy. Cardiovasc. Res. *93*, 320–329.

Wang, R.C., Wei, Y., An, Z., Zou, Z., Xiao, G., Bhagat, G., White, M., Reichelt, J., and Levine, B. (2012). Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. Science *338*, 956–959.

Wang, X., Wang, X.L., Chen, H.L., Wu, D., Chen, J.X., Wang, X.X., Li, R.L., He, J.H., Mo, L., Cen, X., et al. (2014). Ghrelin inhibits doxorubicin cardiotoxicity by inhibiting excessive autophagy through AMPK and p38-MAPK. Biochem. Pharmacol. *88*, 334–350.

Wauson, E.M., Zaganjor, E., Lee, A.Y., Guerra, M.L., Ghosh, A.B., Bookout, A.L., Chambers, C.P., Jivan, A., McGlynn, K., Hutchison, M.R., et al. (2012). The G protein-coupled taste receptor T1R1/T1R3 regulates mTORC1 and autophagy. Mol. Cell *47*, 851–862.

Wauson, E.M., Dbouk, H.A., Ghosh, A.B., and Cobb, M.H. (2014). G protein-coupled receptors and the regulation of autophagy. Trends Endocrinol. Metab. *25*, 274–282.

Wei, Y., Zou, Z., Becker, N., Anderson, M., Sumpter, R., Xiao, G., Kinch, L., Koduru, P., Christudass, C.S., Veltri, R.W., et al. (2013). EGFR-mediated Beclin 1 phosphorylation in autophagy suppression, tumor progression, and tumor chemoresistance. Cell *154*, 1269–1284.

Wieman, H.L., Wofford, J.A., and Rathmell, J.C. (2007). Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. Mol. Biol. Cell *18*, 1437–1446.

Williams-Bey, Y., Boularan, C., Vural, A., Huang, N.N., Hwang, I.Y., Shan-Shi, C., and Kehrl, J.H. (2014). Omega-3 free fatty acids suppress macrophage inflammasome activation by inhibiting NF-κB activation and enhancing autophagy. PLoS ONE *9*, e97957.

Xu, L., Kanasaki, M., He, J., Kitada, M., Nagao, K., Jinzu, H., Noguchi, Y., Maegawa, H., Kanasaki, K., and Koya, D. (2013). Ketogenic essential amino acids replacement diet ameliorated hepatosteatosis with altering autophagy-associated molecules. Biochim. Biophys. Acta *1832*, 1605–1612.

Yang, W., Hong, Y.H., Shen, X.Q., Frankowski, C., Camp, H.S., and Leff, T. (2001). Regulation of transcription by AMP-activated protein kinase: phosphorylation of p300 blocks its interaction with nuclear receptors. J. Biol. Chem. *276*, 38341–38344.

Yang, L., Li, P., Fu, S., Calay, E.S., and Hotamisligil, G.S. (2010). Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell Metab. *11*, 467–478.

Ye, J., Kumanova, M., Hart, L.S., Sloane, K., Zhang, H., De Panis, D.N., Bobrovnikova-Marjon, E., Diehl, J.A., Ron, D., and Koumenis, C. (2010). The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation. EMBO J. *29*, 2082–2096.

Yuan, H.X., Russell, R.C., and Guan, K.L. (2013). Regulation of PIK3C3/VPS34 complexes by MTOR in nutrient stress-induced autophagy. Autophagy *9*, 1983–1995.

Zhang, M., and Schekman, R. (2013). Cell biology. Unconventional secretion, unconventional solutions. Science *340*, 559–561.

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