CELL BIOLOGY

Retrograde signaling from autophagy modulates stress responses

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Macroautophagy is a process in which cytoplasmic components, including whole organelles, are degraded within lysosomes. Basally, this process is essential for homeostasis and is constitutively functional in most cells, but it can also be implemented as part of stress responses. We discuss findings showing that autophagy proteins can modulate and amplify the activities of transcription factors involved in stress responses, such as those in the p53, FOXO, MiT/TFE, Nrf2, and NFκB/Rel families. Thus, transcription factors not only amplify stress responses and autophagy but are also subject to retrograde regulation by autophagy-related proteins. Physical interactions with autophagy-related proteins, competition for activating intermediates, and "signalphagy," which is the role autophagy plays in the degradation of specific signaling proteins, together provide powerful tools for implementing negative feedback or positive feed-forward loops on the transcription factors that regulate autophagy. We present examples illustrating how this network interacts to regulate metabolic and physiologic responses.

Introduction

Macroautophagy (hereafter referred to as autophagy) is a catabolic process that sequesters cytoplasmic elements in double-membrane vacuoles for degradation in lysosomes. Targets of autophagy can be specific proteins, nonspecific protein aggregates, or whole organelles, such as dysfunctional mitochondria. Basal autophagy is constitutive in most cell types (*1–3*), and it is estimated that basal autophagy is responsible for the catabolism of 1 to 1.5% of cellular proteins per hour even under nutrient-rich conditions (*4*). However, because cell survival is constantly threatened by variations in both internal and external conditions, autophagy programs must be able to respond appropriately to stress. Stresses, such as nutrient or growth factor starvation, metabolic imbalance, hypoxia, oxidative stress, genotoxic stress, or unfolded protein stress all affect autophagy and induce adjustments, initially through posttranslational modifications of the autophagy-related (ATG) proteins but eventually of transcription factors, such as those in the p53, forkhead box O (FOXO), microphthalmia transcription factor (MiT/TFE), nuclear factor erythroid-derived 2–related factor 2 (Nrf2), and nuclear factor κ-light chain enhancer of activated B cells (NFκB)/reticuloendotheliosis (Rel) families (*2, 5–9*).

The identification of the *ATG* genes in yeast and in mammals has provided the impetus for a molecular understanding of autophagy (*4, 10*). Autophagy functions have been evolutionarily conserved from *Saccharomyces* to man. There are now more than 30 recognized *ATG* genes, some of which bear individual names for historical reasons, such as *Beclin 1* (which encodes Bcl-2–interacting coiled-coil protein 1), *SQSTM1* (which encodes sequestosome1 and is also known as p62), or *FIP200* (which encodes focal adhesion kinase family–interacting protein of 200 kDa). The proteins encoded by *ATG* genes are required for autophagy. The canonical autophagic pathways (*10–13*) and noncanonical pathways that resemble or overlap with autophagy (*14–16*) have been characterized in excellent reviews. An overview of the principal events and players in autophagy is presented here as a guide for further discussion (Fig. 1).

Autophagy is a part of a complex network that brings together the regulation of stress responses, metabolic oversight, and cell proliferation. This network includes interactions that connect many familiar, linear pathways (*6–9, 11, 12*). An integrated image showing how the separate

pathway fragments fit together in a whole network is an important goal for future research because there is clinical potential in targeting events in autophagy, especially for treatment of neurodegenerative and cardiovascular diseases or cancer. Various retrograde interactions between ATG proteins and transcription factors already provide hints about how this network may work and will be the subject of this Review.

Stresses Trigger Autophagy

mTORC1 has a central role in the regulation of autophagy

Autophagy is a specific, modulated response that is triggered by stresses, such as nutrient starvation, growth, or survival factor deprivation, or the hyperproliferative stimulus induced by oncogenes such as activated Ras (*17–20*). An ultimate target in all forms of stress is the multiprotein mechanistic target of rapamycin complex 1 (mTORC1), a master regulator of cell growth that integrates inputs from at least five pathways: growth factor stimulation, DNA damage, energy status [the ratio of adenosine 5′-triphosphate (ATP) to adenosine 5′-monophosphate (AMP)], amino acid sufficiency, excessive concentrations of reactive oxygen species (ROS), and oxygen availability (Fig. 2) (*21–23*). mTORC1 consists of the serine/threonine protein kinase TOR and several regulatory subunits, including the scaffold protein Raptor. mTORC1 promotes synthesis by phosphorylating numerous targets, including regulators of translation, such as the ribosomal S6 kinase and 4E-BP1, which binds to the initiation factor eIF4E. However, mTORC1 simultaneously inhibits autophagy by directly phosphorylating and inactivating ULK1/2 and ATG13 in the autophagy initiation complex (*22*). Raptor mediates the binding of a fraction of cellular mTORC1 to Rheb (*Ras* homolog enriched in the brain) in association with small guanosine triphosphatases (GTPases) of the Rag family in the Ragulator complex (*22*). These small GTPases are located at the lysosomes (Fig. 2), which thus serve as a critical platform to regulate mTORC1 activity (*24–26*). mTORC1 is active while tethered to the lysosomes in association with Rheb and Rag proteins, but because Rag GTPases bind mTORC1 based on information about the intralysosomal concentrations of amino acids communicated by the proton pump channel vacuolar H+-adenosine triphosphatase (V-ATPase) (*27*), mTORC1 is released, displaced from the lysosome, and inactivated immediately under conditions of amino acid insufficiency (*25, 27*).

As with Rheb- and Rag dependent monitoring of amino acid availability, other inputs on mTORC1 also require recruitment of the tuberous

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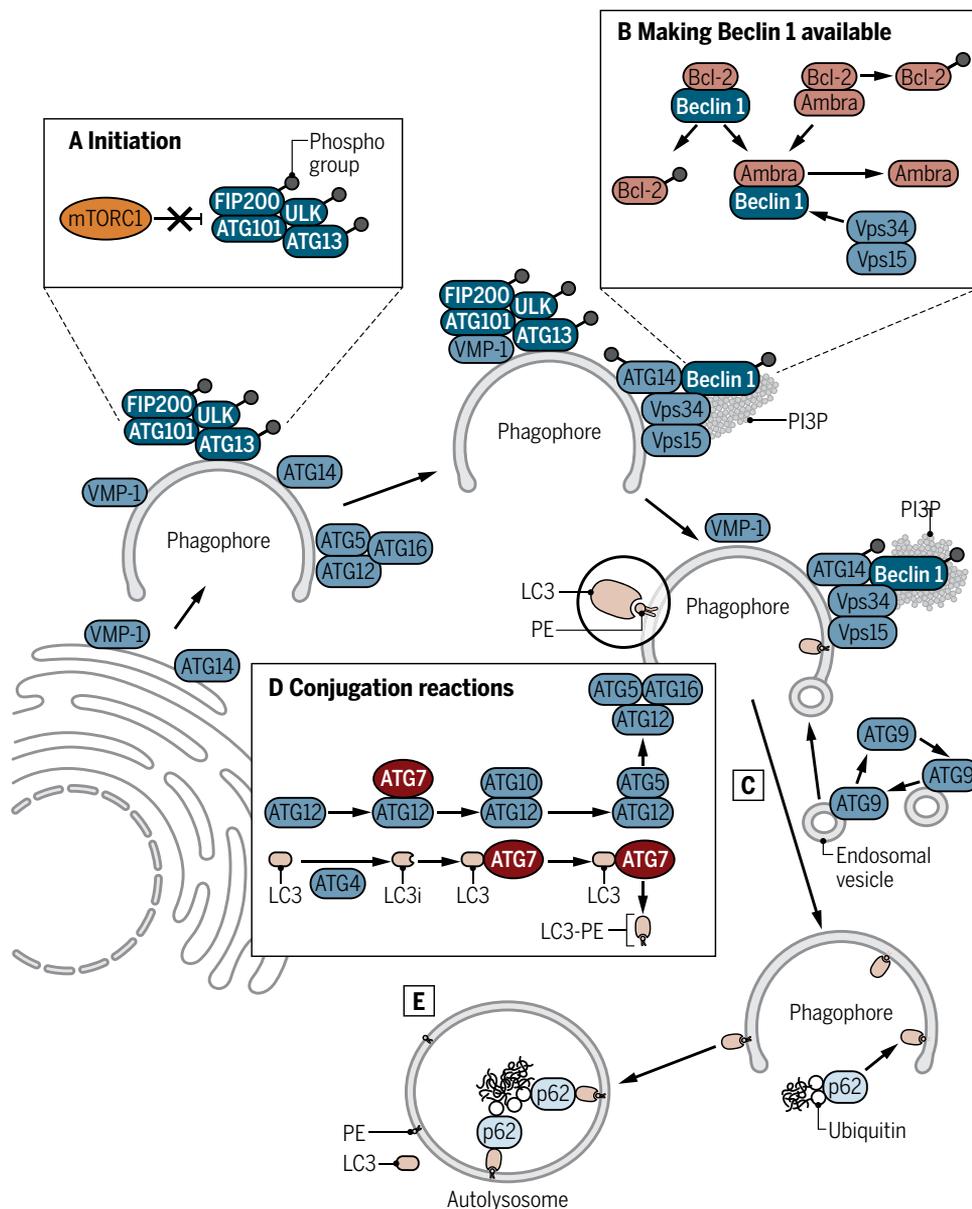*Corresponding author. Email: hus@pki.unibe.ch

Fig. 1. The canonical autophagic pathway carries out the degradation of selected elements as well as that of bulk cytoplasm. (**A**) After mTORC1 is inactivated, its inhibition of the initiation complex comprising the protein kinases unc-51– like kinases 1 and 2 (ULK1/2), ATG13, ATG101, and FIP200 is lifted. A nascent phagophore membrane carrying the endoplasmic reticulum–associated proteins ATG14 and vacuolar membrane protein 1 (VMP1) is formed. (**B**) ULK1/2 phosphorylates ATG14, recruiting Beclin 1 from its cytosolic interaction partners, such as Ambra and Bcl-2. (**C**) Vps34 and Vps15 are recruited to Beclin 1 and form a phosphatidylinositol 3-kinase (PI3K) complex that produces a phosphatidylinositol 3-phosphate (PI3P) cloud locally. PI3P promotes ATG9-dependent membrane acquisition from endosome vesicles. (**D**) PI3P also activates the ubiquitin-like conjugations, initially producing ATG12-ATG5-ATG16 and then LC3-PE (LC3-phosphatidylethanolamine). ATG12-ATG5-ATG16 is present at an early stage of phagophore formation even before full activation of PI3K. Note that the association of ATG12-ATG5-ATG16 and the PI3K complex with the phagophore is transient and that LC3 on the cytoplasmic side of the mature autophagosome is cleaved away. p62 associates with polyubiquitinated protein aggregates and binds to LC3. These p62 interactions allow the phagophore to engulf cytosolic elements, grow, and finally close. (**E**) This forms the autophagosome, which fuses with a lysosome, allowing degradation of the sequestered material.

activity of TSC1/2 to the guanosine di-phosphate (GDP)–bound form (Fig. 2), mTORC1 is inactivated and displaced (24, 25). However, because TSC1/2 is under the subtle and varied control of the growth factor receptor–PI3K–AKT pathway (Fig. 2), its activity is inhibited by AKT-mediated phosphorylation (22, 23). In contrast, other signals acting on the AMP-dependent protein kinase (AMPK), such as low oxygen tension, low energy status, or increased concentrations of ROS, can trigger activation of TSC1/2 (Fig. 2), thus provoking conversion of Rheb to the GDP-bound form with concomitant mTORC1 inactivation, its displacement from the lysosome, and the initiation of autophagy (22, 25).

AMPK is a complex of three proteins and is key in regulating energy status through continuous monitoring of the [AMP]/[ATP] ratio. AMPK can integrate information from various pathways (Fig. 2). Glucose starvation, which results in an increased [AMP]/[ATP] ratio, is one signal that stimulates AMPK to phosphorylate TSC1/2. Increased abundance of SESN1 and SESN2 proteins (also designated Sestrin 1 and Sestrin 2) after their transcription by p53 in response to stress (Fig. 2) also leads to AMPK activation (28, 29), as does its phosphorylation by p53-dependent activation of liver B1 kinase (LKB1) (30, 31). Moreover, oxidized ataxia-telangiectasia–mutated (ATM) kinase dimers formed in the cytoplasm in the presence of ROS (32) contribute to activating the kinase activity of AMPK toward TSC1/2.

Autophagic responses are induced by many kinds of stress other than just starvation. However, because almost all stress responses overlap largely with the phenotype seen after starvation, the induction of autophagy is a central feature (33, 34). This is because, in practically all forms of stress, one common denominator is the increased formation of ROS, which is released by the mitochondria as a consequence of almost any dysfunction, metabolic deficiency, or imbalance and triggers autophagy (34–36).

sclerosis complex (TSC) containing TSC1 and TSC2 (25) to the Rag proteins at lysosomes. The TSC1/2 complex is a GTPase-activating protein (GAP); its association with the Rag GTPases brings it into close proximity with Rheb (25), the guanosine 5′-triphosphate (GTP)–bound form that is essential for mTORC1 activity. When Rheb-GTP is converted by the GAP

The DNA damage response triggers p53 activation

The DNA damage response (DDR) triggers multiple pathways focused on the AMPK complex, which leads to the initiation of autophagy (Fig. 2), but potentially also to apoptosis. The DDR is a prototypical stress response made up of several tasks: sensing DNA damage, repairing
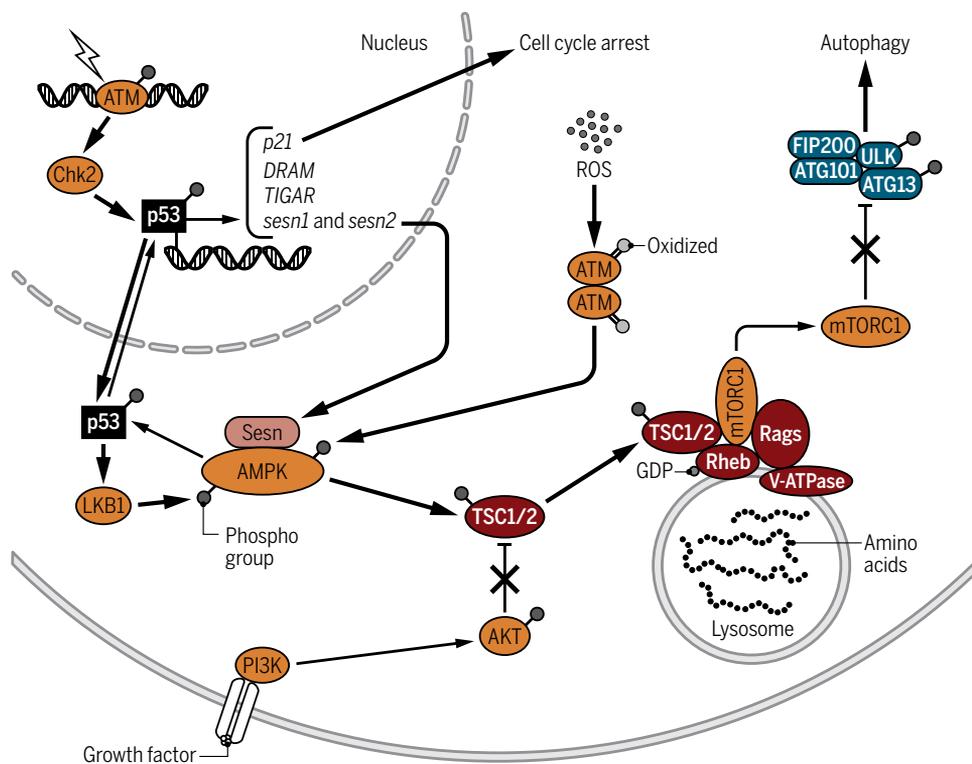
Fig. 2. Several pathways trigger autophagy after DNA damage. The pathways activated after DNA damage or oxidative stress are shown with thicker arrows. DNA double-strand breaks activate ATM to phosphorylate Chk2, which then activates p53. p53 transactivates $p21^{CDKN1A}$ to trigger cell cycle arrest, whereas other p53 targets encode proautophagic proteins such as SESN1, SESN2, and DRAM. Activated p53 is also translocated to the cytoplasm, where it activates LKB1 to phosphorylate AMPK. ROS accumulation also causes oxidized ATM dimers to activate AMPK. After glucose starvation, AMPK can also send a retrograde stress signal by phosphorylating p53. mTORC1 responds to multiple inputs in which AMPK plays a central role. AMPK determines the activation of TSC1/2, which causes Rheb inactivation. Inactive Rheb-GDP enforces mTORC1 inactivation and displacement from the lysosome, thus relieving its inhibitory block on autophagy. In unstressed cells, growth factor stimulation keeps TSC1/2 inactive because of a different phosphorylation event mediated by AKT. In addition, amino acid availability is monitored by Rag proteins, and lack of essential amino acids causes mTORC1 displacement and inactivation.

DNA damage, and making physiological corrections, such as arresting proliferation and responding to increased metabolic demands with autophagy (37). The response to potentially lethal DNA double-strand breaks caused by ionizing radiation and by some chemotherapy drugs follows a typical pattern. Briefly, these DNA strand breaks lead to the activation of the large (350 kDa) serine/threonine protein kinase ATM, which serves as a master regulator and transducer (Fig. 2) for DNA repair (37) but also participates in the stress response. ATM is activated by the formation of the Mre11-Rad50-Nbs1 complex bound to the ends of the DNA breaks and is essential for repair (38). Moreover, poly(adenosine 5′-diphosphate-ribose) polymerase 1 (PARP1) activation at the DNA strand breaks produces chains of PAR on proteins, including PARP1 itself, and marks the break sites. PARP1 activation can induce a bioenergetic collapse due to the massive ATP and NAD$^+$ (nicotinamide adenine dinucleotide) consumption resulting from PAR synthesis at multiple sites of DNA damage (37, 39) and also partly because PAR binds to and inhibits hexokinase (40), hence inhibiting the first step in glycolysis. An increase in the [AMP]/[ATP] ratio and in mitochondrial ROS output would then contribute to AMPK activation, followed by mTORC1 inactivation and autophagy just as in glucose starvation. Moreover, there is evidence that, unlike with glucose starvation, oxidative stress and ROS can increase ULK1 abundance in addition to that induced by autophagy. Increased abundance of ULK1 leads to its localization in the nucleus where it

binds and activates PARP1, eliciting just such a bioenergetic collapse (41).

After its activation, monomerization, and autophosphorylation at several sites, ATM initiates a phosphorylation cascade in the nucleus. ATM phosphorylates numerous targets (Fig. 2) on DNA, such as histone 2AX, and on checkpoint 2 protein kinase (Chk2), which, in turn, rapidly phosphorylates and activates p53 (42, 43). Although phosphorylation of human p53 at Ser^{20} by Chk2 is important for its transactivation of $p21^{CDKN1A}$ (which encodes the cyclin-dependent kinase inhibitor 1A) and other stress response targets (44), p53 tetramerization is also essential for its activation (45, 46).

After glucose starvation, p53 is also subject to retrograde phosphorylation and activation by AMPK, which is normally downstream of p53 (Fig. 2) (47–49). The tumor suppressor protein p53 is of particular importance in all forms of stress, partly because it is a transcription factor responsible for transactivation of many genes important in cellular metabolism, autophagy, and apoptosis (50–53) and partly because it has important functions as an effector in the cytoplasm. After stress, transactivation of $p21^{CDKN1A}$ by p53 (Fig. 2) contributes to G$_1$ and G$_2$ cell cycle arrest, whereas p53 transactivation of other genes, such as *Sesn1* and *Sesn2*, promote autophagy and trigger a conservative, catabolic metabolism by activating AMPK (28, 54). Similarly, p53 transactivates the lysosomal

protein damage-regulated autophagy modulator (DRAM) (55, 56), which contributes to both autophagy and apoptosis, depending on context. Another p53 transcriptional target, tumor protein p53–induced glycolysis and apoptosis regulator (TIGAR), inhibits autophagy, reducing ROS concentrations by increasing flux through the pentose phosphate pathway and increasing NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) generation to produce the reduced glutathione needed to scavenge ROS (50). Finally, among the other genes transactivated by p53 under stress conditions, several, such as *Puma*, *Noxa*, and *Bax*, encode proapoptotic factors (2, 35).

Under stress conditions, phosphorylated, activated p53 can also be found in the cytoplasm (Fig. 2), where it acts not only as a transcription factor but also as a positive effector for autophagy, complexing with and activating LKB1 and triggering its phosphorylation of AMPK (30, 31). Furthermore, p53 activated under stress conditions can also take on a direct apoptogenic role in the mitochondria (not shown in Fig. 2) (57–59). However, under basal, unstressed conditions, cytoplasmic p53 plays an entirely different role as an inhibitor of autophagy (60–62), which is due to its association with FIP200, an essential component of the ULK1/2 complex responsible for autophagy initiation (Fig. 1) (62, 63). Under stress-free conditions, cytoplasmic p53 protein tonically inhibits basal autophagy. Thus, experimental p53 depletion or treatment of unstressed cells with the p53 transcriptional inhibitor pifithrin-α causes an increase

in basal autophagy (60, 61). Thus, p53 has a dual role because it not only stimulates autophagy in response to stress but also inhibits basal autophagy tonically (60).

ATG Proteins Act in a Retrograde Fashion on Transcription Factors

ATG7 is an essential accessory protein for p53-mediated transactivation of $p21^{CDKN1A}$

If one takes the view that transcription factors act upstream of their target genes, then retrograde signaling means that these gene products themselves act on and influence transcription factor function. Basal autophagy is believed to be maximal during the G_1/S phase of the cell cycle and is inhibited during the G_2/M phase because the anabolic status of growing cells is inconsistent with high amounts of autophagy (64). Unsurprisingly, the fraction of wild-type mouse embryonic fibroblasts (MEFs) entering into S phase decreases by 60% within 3 hours of glucose or amino acid starvation (35). In comparison, this decrease is only 20% in Atg7 knockout MEFs. Whereas starvation increases the abundance of the cyclin-dependent kinase inhibitor 1A (p21) in wild-type MEFs, this increased abundance does not occur in starved Atg7 knockout MEFs. However, the abundance of cyclin-dependent kinase inhibitor 1B protein (p27), which is transcribed by the FOXO family of transcription factors, is comparable between the two cell types. The explanation is that in wild-type MEFs, endogenous ATG7 is present together with p53 on the $p21^{CDKN1A}$ gene promoter (Fig. 3 and Table 1) (35). The same complex is found in both the cytoplasm and nucleus, and its abundance increases after nutrient starvation as cells enter growth arrest.

Other ATG proteins, such as Beclin 1 or ATG5, do not appear to physically associate with p53. However, after starvation, a subset of p53-regulated proapoptotic genes, namely Puma, Noxa, and Bax, show enhanced abundance at the RNA and protein levels in both Atg7 and Atg5 knockout MEFs as compared with wild-type cells, suggesting that these ATGs normally inhibit the transactivation of these proapoptotic genes (35). ATG7 seems to promote p53 tetramerization in a manner that does not require the E1 ligase–like enzymatic activity that is needed for autophagy. ATG7 mutants lacking the C-terminal cysteine active site and that are thus nonfunctional for conjugation reactions in autophagy can still bind to p53 and promote $p21^{CDKN1A}$ transactivation. Together, these findings indicate that ATG7 acts as an accessory protein for p53 in the process of the $p21^{CDKN1A}$ gene transactivation (35), much like other accessory proteins involved with other p53 targets (65, 66).

Ectopic ATG5 expression initiates a stress response

The goal of most anticancer therapy protocols is to induce irreparable DNA damage in tumor cells. These protocols inevitably produce a DDR with activation of ATM, p53, and various stress responses (67, 68). Low, sublethal doses of the topoisomerase II inhibitor etoposide or the DNA cross-linking agent cisplatin induce marked

increases in the abundance of both ATG5 and p53, as well as activation and phosphorylation of the latter (69); cell cycle arrest occurs, but without apoptosis. These chemotherapeutics trigger a rapid increase in autophagic flux that is secondary to increases in the abundance of p53 and ATG5 because p53 and ATG5 abundance increases even in the presence of the autophagy blocker and the PI3K type 3 inhibitor 3-methyladenine. Blocking autophagic flux with 3-methyladenine, however, massively increases caspase-dependent apoptotic cell death (69), suggesting that autophagy can be a trigger for apoptosis.

Surprisingly, ectopic expression of ATG5 in cells without drug treatment also stimulates increases in the abundance, phosphorylation, and activation of p53 with concomitant $p21^{CDKN1A}$ transactivation (Fig. 3 and Table 1) (69, 70). Thus, ectopically expressed ATG5 induces not only autophagy but also a stress response, implying that ATG5 may affect AMPK because p53 can be activated upstream of AMPK (Fig. 2) (48). How this happens is unclear, but a feed-forward loop between p53-mediated transactivation of sesn1 and sesn2, followed by AMPK potentiation and additional p53 activation, would be a possible mechanism (Fig. 3) (47–49). In contrast, ectopically expressed Beclin 1 induces neither p53 activation nor growth arrest (69).

In addition, ATG5 overexpression culminates in mitotic catastrophe (69), with multinucleation or enlarged abnormal nuclei, a consequence of its localization in the nucleus (69, 71). This localization is consistent with the presence of a leucine-rich region that acts as a nuclear export signal in ATG5 (69, 71), similar to that found also in Beclin 1 (72). Both Beclin 1 (73) and ATG5 (69, 71) physically associate with survivin (also known as BIRC5), an essential component of the chromosome passenger complex. The competition between nuclear ATG5 and Aurora B for survivin/BIRC5 affects the recruitment of the chromosome passenger complex to centromeres during mitosis (69, 71). In cells that ectopically express ATG5, Aurora B is present at centromeres only in reduced amounts during prometaphase. Survivin is present at centromeres at normal amounts but is retained there during anaphase, unlike normal cells that show survivin mainly at the central spindle during anaphase (69).
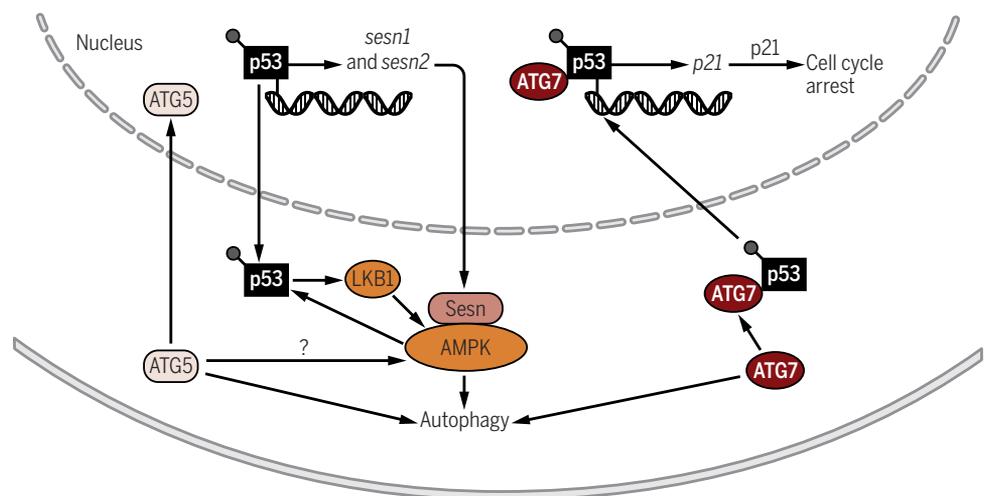

Fig. 3. ATG5 and ATG7 affect the p53 pathways in stress responses. ATG7 is an essential accessory protein for the activation of p53-dependent transactivation of $p21^{CDKN1A}$, leading to cell cycle arrest. ATG7-p53 complexes are found in both cytoplasm and nucleus of normal cells. Both ectopically expressed ATG7 and ATG5 induce autophagy; ATG5 also increases p53 abundance, activation, and transactivation of $p21^{CDKN1A}$. Question mark (?) represents a hypothetical stimulatory loop that may be responsible for such p53 activation after ectopic ATG5 expression. This proposal is based on the positive feed-forward loop in which AMPK phosphorylates p53, which leads to p53-dependent transactivation of sesn1 and sesn2 and a resulting potentiation of AMPK activation. Furthermore, ATG5 is abundant in the nucleus.

Table 1. ATG proteins act in a retrograde fashion on transcription factors.

ATG	Retrograde function	References
Beclin 1	Autophagy and NFκB/IKK activation are interdependent.	(104, 105)
ATG5	Ectopic ATG5 initiates a stress response involving p53 induction and activation and, owing to its nuclear localization, elicits mitotic catastrophe.	(69–71)
ATG5 + ATG9	In mitophagy, TFEB requires ATG5 and ATG9 for nuclear translocation.	(8, 98, 99)
ATG7	ATG7 is an accessory protein for p53-dependent transactivation of $p21^{CDKN1A}$.	(35)
	ATG7 exerts negative feedback regulation on FOXO1.	(79–81)
LC3	In melanocytes, MITF abundance and its regulation of melanin and tyrosinase synthesis depend on LC3.	(101)
p62/SQSTM1	Selective p62-mediated autophagy of KEAP1 creates a feedback loop for Nrf2 amplification.	(5, 89–91)
ULK1	Nuclear ULK1 activates PARP1, eliciting a bioenergetic crisis and cell death.	(41)
UVRAG	UVRAG functions in DNA repair by triggering nonhomologous end joining (NHEJ).	(106)

Disturbances in cytokinesis often lead to genetically abnormal cells, mitotic catastrophe, polyploidy, aneuploidy, imbalances in gene dosage, and in some fraction of cells, to tumorigenesis (74).

ATG7 mediates negative feedback regulation of FOXO1

FOXO transcription factors transactivate many genes involved in autophagy (ULK1, LC3, Gabarapl1, ATG5, ATG8, ATG12, ATG14, Beclin 1, and Bnip3), which enable survival under stress conditions (75–77). ATG7 binds to acetylated FOXO1 in the cytoplasm (78–80), forming ATG7/FOXO1 complexes that stimulate autophagy (Fig. 4 and Table 1). The accumulation of acetylated FOXO1 in the cytoplasm occurs when the deacetylase Sirt2 dissociates from FOXO1 because of oxidative stress. In this role, FOXO1 acts as a cytoplasmic effector, not as a transcription factor, because FOXO1 mutants defective for transcription factor activity retain the ability to bind ATG7 and initiate autophagy (79). Presumably, the induction of autophagy results from the activation of ATG7 in the ubiquitination-like conjugation reactions, leading to formation of ATG5-ATG12 complexes and LC3-phosphatidylethanolamine, which are essential for the formation and closure of the autophagosome. At the same time, FOXO1 is retained in the cytoplasm in a transcriptionally inactive state because it is complexed to ATG7 (Fig. 4). Hence, this phenomenon represents a kind of negative feedback, preventing FOXO1 from acting as a transcription factor. Furthermore, this cytoplasmic FOXO1 function also has a tumor-suppressive effect due to the induction of autophagic cell death (79, 81). FOXO3 transcriptionally represses the expression of the gene encoding FOXO1 (Fig. 4), and FOXO3 knockdown is followed by a FOXO1-dependent increase in autophagy (82).

p62-dependent selective autophagy creates a feedback loop for Nrf2 amplification in stress response pathways

Autophagy is vitally important for the genetic stability of the cell. Loss of functional autophagy causes genetic instability, owing to the accumulation of toxic protein aggregates containing p62 (also known as SQSTM1), which correlate with dysfunctional mitochondria and increased ROS production (83, 84). p62 is not only an autophagy receptor and adaptor but also a substrate that must be removed and degraded with the cargo through autophagy. Enforced p62 accumulation induces ROS production, DNA damage, aneuploidy, and tumorigenesis (84). Furthermore, inhibition of
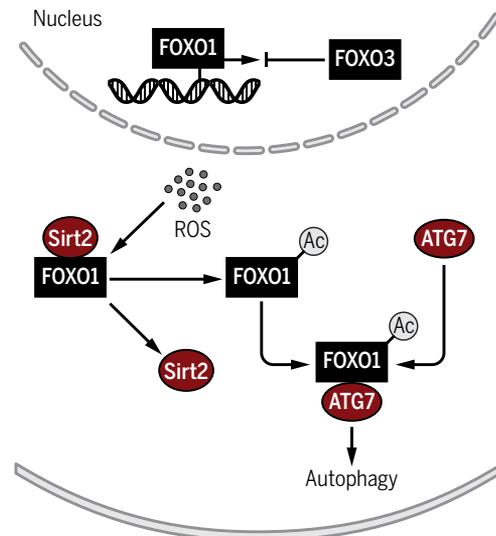

Fig. 4. ATG7 mediates negative feedback regulation of FOXO1. The transcription factor activity of FOXO3 antagonizes *FOXO1* expression in the nucleus, thus suppressing autophagy. However, after inactivation of the deacetylase Sirt2 caused by oxidative stress, acetylated (Ac) cytoplasmic FOXO1 accumulates in the cytoplasm and complexes with ATG7. FOXO1 is then retained in the cytoplasm, and autophagy is induced by the accumulating acetylated FOXO1/ATG7 complex. This mechanism for autophagy induction suppresses tumor xenograft growth in nude mice in an autophagy-dependent manner.

autophagy leads to increased proteasomal activity and results in enhanced degradation and reduced abundance of Chk1, an essential factor for homologous recombination, and to a corresponding deficit in DNA repair of double-strand breaks (85).

The selectivity of autophagy for particular protein targets explains some important feedback pathways. Autophagy is critical for the disposal of the dishevelled (Dvl) protein under conditions of nutrient stress; hence, autophagic activity inversely correlates with the cytoplasmic abundance of Dvl and the intensity of Wnt signaling as well (86). The term "signalphagy" coined by Belaid et al. refers to dedicated, selective autophagy processes that regulate the abundance of particular signaling proteins (87). For

example, whereas the inactive form of Ras homolog family member A (RHOA) is proteasomally degraded, active RHOA-GTP is rapidly and selectively degraded by autophagy (88). The basis for this selective degradation is the specificity of p62 and related autophagy receptor/adaptor proteins, which mediate the association with lipidated LC3 at the developing autophagosome for individual targets (88).

p62 is encoded by *SQSTM1*, which is a target for transactivation by the transcription factor Nrf2 (5). *SQSTM1* transactivation by Nrf2 is induced as a part of the overall antioxidant and detoxification response, which is seen, for example, under conditions of increasing intracellular ROS concentrations. p62 plays an essential retrograde role in controlling the abundance of Nrf2, which is important for maintaining the redox balance in the cell (Fig. 5 and Table 1) (5, 89). The transcription factor Nrf2 is subject to ubiquitination through the binding of kelch-like epichlorohydrin-associated protein 1 (Keap1), a component of a Cullin-3–type ubiquitin ligase, and is retained in the cytoplasm and targeted for degradation in the 26S proteasome (Fig. 5). Because p62 serves as a specific autophagy receptor for Keap1 (Fig. 5) (89), this mechanism provides a feed-forward loop to maintain appropriate abundance of Nrf2 (5), which is, however, conditional on a functional autophagic flux.

This loop concept leaves us asking how p62 function is regulated. One way is its transcriptional up-regulation as a target of Nrf2-dependent transcription. However, what if p62 abundance is low and Nrf2 is entirely bound up by Keap1 and subject to degradation? Phosphorylation of p62 on Ser[351] promotes increased affinity of p62 for Keap1 with concomitant degradation through autophagy (90). Furthermore, SESN2 also promotes the interaction between p62 and Keap1 and autophagic degradation of Keap1 (91). Thus, p62 activation by phosphorylation and/or by SESN2 binding effectively limits Keap1 abundance and stabilizes Nrf2, thereby enabling transcription of the antioxidant program as needed (Fig. 5).

MiT/TFE transcription factors depend on ATG functions

The lysosome plays a central role in the regulation of autophagy and metabolism (Fig. 2) (24–26). The lysosome is integrally involved in the initiation of autophagy, and of course, lysosomal degradative capacity must also be regulated according to demand. Lysosome biogenesis is regulated by the MiT/TFE transcription factor family, basic helix-loop-helix leucine zipper proteins binding to the E-box core sequence (CANNTG) (92). Members of this family associate with the Rag small GTPase proteins and are phosphorylated by active mTORC1 on the lysosomal outer surface, which results in their binding to the chaperone 14-3-3 and sequestration in the cytosol. However, in response to nutrient starvation, inactivation of mTORC1 is rapidly followed by transcription factor EB (TFEB) dephosphorylation, its translocation into the nucleus, and transactivation of numerous genes for autophagy and for lysosomal biogenesis (Fig. 6 and Table 1) (93, 94). These include genes that encode lysosomal acid lipase, the proton pump V-ATPase, p62, and ATG9 (26, 93).

TFEB and other MiT/TFE transcription factors are closely integrated with the process of autophagy (8). For mitophagy, a special form of autophagy modified for the lysosomal degradation of dysfunctional mitochondria, TFEB translocation into the nucleus requires the functions of both ATG5 and ATG9 (Fig. 6 and Table 1). Because mitochondria are comparatively large organelles, their degradation requires substantial increases in autophagic and lysosomal capacity. Mitophagy is a form of selective autophagy and an essential mitochondrial quality control function (95). After loss of the mitochondrial membrane potential or accumulation of misfolded proteins in a mitochondrion, phosphatase and tensin homolog–induced kinase 1 (PINK1) accumulates on the outer mitochondrial

membrane where it recruits the E3 ubiquitin ligase Parkin from the cytosol. PINK1 activates and phosphorylates Parkin so that it ubiquitinates proteins on the outer mitochondrial membrane, thereby marking the damaged organelle for binding by autophagy receptors/adaptors (Fig. 6). Parkin activation takes place as a two-step event: PINK1 phosphorylates Parkin on Ser[65] in its ubiquitin-like domain and phosphorylates its bound ubiquitin, thereby activating its ligase function (96, 97). Several autophagy receptors/adaptors, such as p62 and optineurin, can bind to the ubiquitin chains on damaged mitochondria; however, NDP52 and optineurin, not p62, are the primary receptors/adaptors for PINK1- and Parkin-mediated mitophagy, providing the bridge between the mitochondria and lipidated LC3 (98). Furthermore, these receptors/adaptors are responsible for recruiting ULK1/2, DFCP1, and WIPII (not shown in Fig. 6) to early phagophores that form adjacent to the mitochondria (98).

TFEB activation, translocation, and functional transactivation of needed target genes requires Parkin, ATG5, and ATG9, thus illustrating the interdependence between the early stages of phagophore construction (99, 100) and the projected transcriptional needs of mitophagy (8). Such interdependence is not seen when TFEB activation occurs after nutrient starvation, a stimulus that requires the biogenesis of autophagosomes and lysosomes to a lesser extent. In this case, TFEB activation, nuclear translocation, and transcriptional activity are independent of ATG5, ATG9, and Parkin (8).
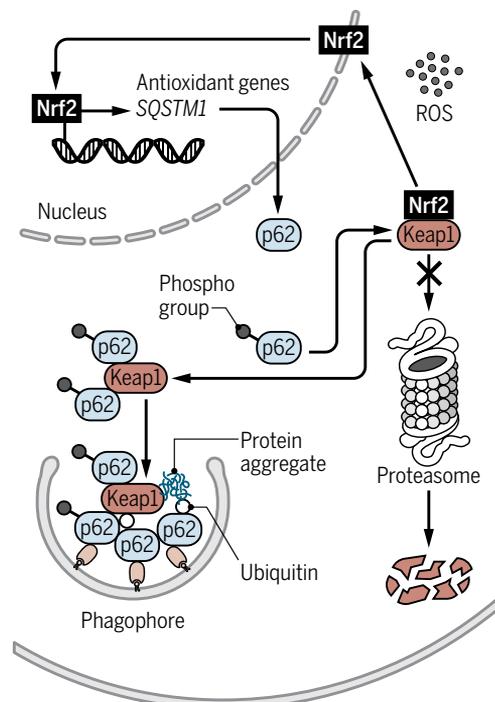

Fig. 5. p62-dependent selective autophagy creates a feedback loop for Nrf2 amplification in stress response pathways by promoting Keap1 degradation. Because *SQSTM1*, the gene for p62, is transactivated by Nrf2, a feed-forward loop is established when ROS concentrations rise. Nrf2 plays an important role in regulating genes that encode antioxidant proteins. Keap1 is a ubiquitin ligase that targets the Nrf2 transcription factor for proteasomal degradation. However, the autophagic cargo receptor/adaptor p62 complexes with Keap1, sequestering it together with aggregates of other ubiquitinated proteins in the developing phagophore for degradation. The affinity of p62 for Keap1 is increased after its activation by SESN2 binding (not shown) or after phosphorylation, thus intensifying the positive feedback loop by increasing the rate of Keap1 degradation.
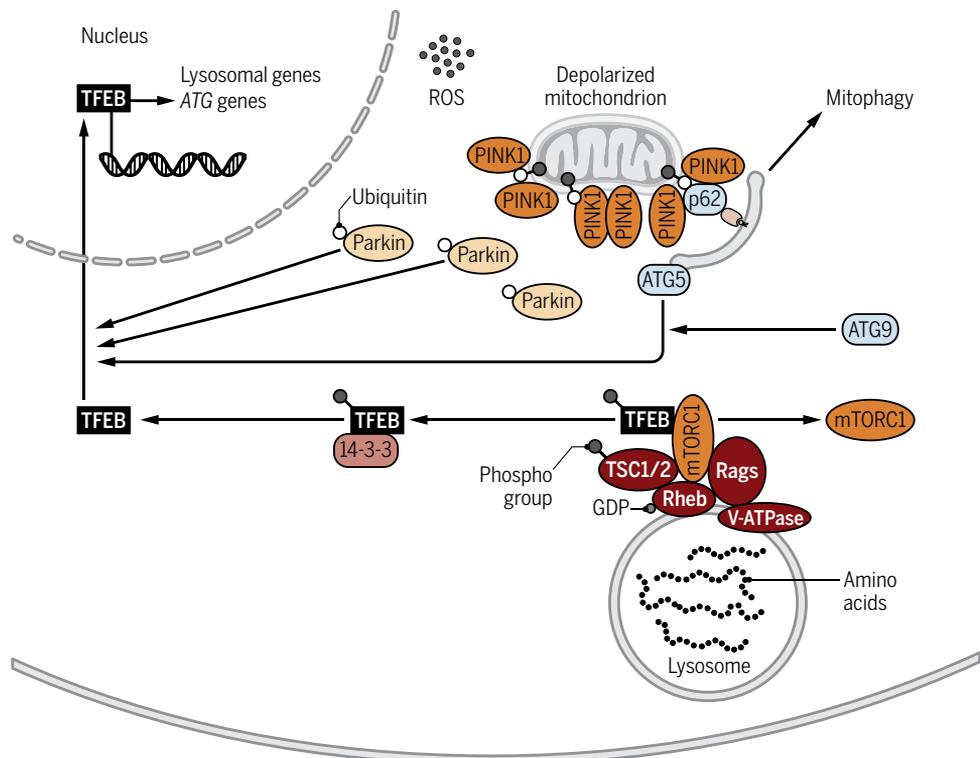
Fig. 6. The TFEB transcription factor requires parkin, ATG5, and ATG9 for regulation of autophagic and lysosomal gene expression in mitophagy. Mitophagy is a massive, selective autophagic degradation that increases demand for both autophagic and lysosomal proteins. The expression of genes encoding these proteins depends on the MiT/TFE family of transcription factors (exemplified here by TFEB). These transcription factors are retained in the cytoplasm with the chaperone 14-3-3 after their phosphorylation by active mTORC1. After mitochondrial dysfunction, with accompanying ROS production, the kinase PINK1 phosphorylates the E3 ubiquitin ligase parkin, recruiting it to the outer mitochondrial membrane where it ubiquitinates numerous mitochondrial surface proteins. These then can serve as targets for autophagy receptors/adaptors, which bridge the targets with LC3 on the phagophore. TFEB activation and translocation into the nucleus take place in a manner dependent on parkin, ATG5, and ATG9 and enables the successful transactivation of numerous lysosomal and autophagy genes. The role of ATG5 and ATG9 in contributing to the formation of the phagophore is the probable mechanism for their essential role in TFEB translocation into the nucleus, although some evidence suggests that both ATGs also enter into the nucleus (8). Glucose deprivation also stimulates TFEB activation and translocation but is not dependent on ATG5 and ATG9 dependence.

LC3 has also been implicated in the regulation of microphthalmia-associated transcription factor (MITF), another member of the MiT/TFE transcription factor family, in the context of melanoma and pigment-rich melanocytic nevi (Table 1). MITF stimulates melanin and tyrosinase abundance, whereby initiation of autophagy by rapamycin increases the abundance of MITF protein. Knockdown of LC3, but not that of Beclin 1 or ATG5, inhibits this increase in MITF abundance and prevents the associated increase in melanogenesis (101). Thus, LC3 regulates MITF abundance by a mechanism independent of its autophagic function.

Autophagy and NFκB/IKK activation are interdependent

NFκB can transactivate many autophagy-relevant genes, including *Beclin 1*. NFκB plays a central role in transactivating genes involved in inflammatory responses, and the general tendency of NFκB-regulated transcription is proautophagic. Signaling pathways leading to NFκB activation also overlap with autophagy (102–104). The inhibitor of nuclear factor κB (IκB) kinase (IKK) brings about the activation of NFκB by committing IκB to proteasomal degradation. For IKK activation, transforming growth factor–β (TGF-β)–activated kinase 1 (TAK1) and its binding partners TAB2 and TAB3 are required. However, because

TAB2 and TAB3 exist as complexes with the autophagy protein Beclin 1, TAK1 and Beclin 1 are competitors for TAB2 and TAB3. Beclin 1 has many other binding partners (a prominent example is the antiapoptotic Bcl-2; see Fig. 1), all of which limit its availability, but upon its release from these complexes, it can initiate autophagy and promote the IKK-dependent activation of NFκB (Fig. 7 and Table 1).

Autophagy requires both the release of Beclin 1 for autophagy and IKK activation. Reciprocally, autophagy must be functional for NFκB activation to occur (105). Stimuli that induce autophagy, such as starvation, or drugs, such as the mTORC1 inhibitor rapamycin or the p53 inhibitor pifithrin-α, also strongly stimulate activation of IKK, translocation of NFκB/p65Rel into the nucleus, and transcriptional activation of NFκB target genes. In wild-type MEFs, the activation of the canonical NFκB pathway must be accompanied by initiation of functional autophagy (105); thus, in *Atg5-* or *Atg7*-deficient MEFs, the NFκB pathway remains largely inactive (Fig. 7). Here again, there is functional interdependence between autophagy and a transcription factor important for stress responses and especially inflammatory responses.

UVRAG represents a hub for different functions regulating autophagy and cancer

Autophagy deficiency causes aneuploidy and oncogenic transformation in cells because of accumulating toxic protein aggregates, dysfunctional mitochondria, and ROS (84). However, autophagy can specifically protect against genomic instability and confer centrosome protection through the ultraviolet (UV) irradiation resistance–associated gene (UVRAG), which was originally isolated in a screen for genes complementing UV sensitivity in xeroderma pigmentosum cells. As part of a complex with Beclin 1 and Vps34, this broadly multifunctional protein (Table 1) behaves as a type 3 PI3K and contributes to the stimulation of autophagy. In the nuclei of irradiated cells, UVRAG is bound to DNA-dependent protein kinase, a key enzyme involved in recognizing sites of DNA damage and in NHEJ (106). Furthermore, UVRAG also participates in centrosome protection because depletion of UVRAG leads to centrosome amplification with consequent malfunctioning of the spindle apparatus and errors in chromosome segregation. These functions of UVRAG are distinct from its role as a Beclin 1 binding partner in autophagy, because mutants that are competent for autophagy can be isolated, but not for genome or centrosome protection and vice versa (106).

UVRAG not only acts early in autophagy initiation as an element of a PI3K complex but also regulates late stages of autophagosome maturation, such as endosome and lysosome fusion events. In this role,
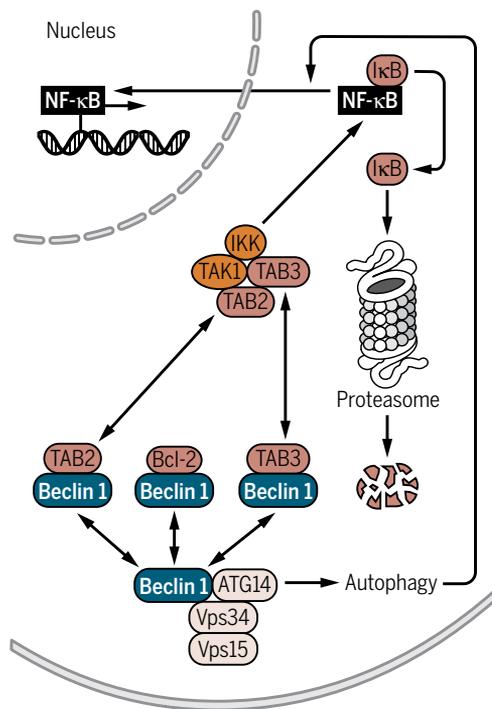
Fig. 7. Reciprocal interdependence of autophagy and NFκB activation. NFκB is normally retained in the cytoplasm and bound to its inhibitor IκB. It is activated by the IKK complex, which can displace IκB for proteasomal degradation, thereby enabling NFκB translocation into the nucleus. For IKK function, however, the TGF-β–activating kinase 1 (TAK1) is required, together with its cofactors TAB2 and TAB3. Because these two cofactors exist as complexes with Beclin 1, which participates in complexes with Bcl-2 and Bcl-X$_L$ as well, NFκB activation can only occur in parallel with autophagy as a consequence of shifts in these equilibria, making TAB2 and TAB3 available for IKK activation, but at the same time releasing free Beclin 1 to initiate autophagy.

UVRAG is again under the control of mTORC1 localized at lysosomes. UVRAG phosphorylation by mTORC1 blocks further autophagosome maturation and fusion with lysosomes, providing an additional level of control on the rate of autophagic degradation (*107*). Thus, mTORC1 not only regulates early stages of autophagy, preventing initiation by phosphorylating ULK1/2, but can also inhibit late autophagosome maturation and lysosomal fusion if the protein kinase activity of mTORC1 is restored, greatly delaying the degradation of autophagy cargoes.

Conclusion

Several lines of evidence indicate that autophagy is a pathway that is closely integrated with the stress response network because, although autophagy is a major functional target of stress responses, it also influences the activity of transcription factors that regulate these responses. We have discussed various ways in which individual ATG proteins can modulate and amplify the activities across the entire stress network. For example, Beclin 1 plays essential roles in many membrane-trafficking pathways (*17*, *18*), competitively binds to various proteins involved in NFκB activation (*104*, *105*), and serves as a balance point between apoptosis and autophagy (*12*). Similarly, ATG5 increases p53 abundance and activation (*69*, *70*), not only initiating a transcriptional stress response together with autophagy but also interacting with survivin to cause chromosome passenger complex dysfunction. In addition, ATG5 can undergo calpain cleavage to produce a proapoptotic fragment that can induce ap-

optosis (*108*, *109*). ATG7 serves as an essential accessory protein required for p53-dependent transactivation of *p21*^{CDKN1A} and growth arrest in DDR (*35*), whereas both ATG5 and ATG7 suppress apoptosis by repressing p53-dependent transactivation of *Puma*, *Noxa*, and *Bax* (*35*). Furthermore, ATG7 interacts with acetylated FOXO1 to induce autophagy (*80*, *81*). Until now, the pieces of the puzzle have fallen into place somewhat coincidentally in the course of investigations into the functions of individual autophagy-relevant proteins. A screen that aims to find autophagy-relevant genes that affect stress response pathways independently of autophagy would be a worthwhile project.

Gaining more knowledge about the "connectedness" of ATG proteins in cellular physiology is important for understanding the complexity of choices available to a cell in combination with autophagy, for example, senescence or apoptosis. This is for two reasons. First, although autophagy is considered a prosurvival mechanism, it can also lead to the so-called type II–programmed cell death, a caspase-independent cell death distinct from apoptosis (*110*). Thus, further study of retrograde autophagy functions could provide insight into the role of autophagy not only as a response needed for survival but also as a trigger for cell death and aging. Second, new therapeutic approaches, especially for neurodegenerative diseases and cancer, may result from this research. However, the vital prerequisite for progress in this direction is understanding how autophagy is embedded in central cellular regulatory pathways, including stress responses.

Unfortunately, the context of cancer illustrates the problems and underlines how difficult it will be to intervene selectively with drugs against the helpful survival facility that autophagy can provide to established tumor cells (*111*, *112*). Although stress response and autophagy are evolutionarily conserved programs that help hinder oncogenesis, they also ensure some degree of chemotherapy resistance in established tumors. Protocols that eliminate autophagy without incapacitating stress responses and increasing the long-term oncogenic risks are not currently available. Therefore, further investigation in this area will help achieve better autophagy-directed cancer therapies in the future.

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