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A GUIDE TO...

A guide to the regulation of selective autophagy receptors

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Autophagy is a highly conserved catabolic process cells use to maintain their homeostasis by degrading misfolded, damaged and excessive proteins, nonfunctional organelles, foreign pathogens and other cellular components. Hence, autophagy can be nonselective, where bulky portions of the cytoplasm are degraded upon stress, or a highly selective process, where preselected cellular components are degraded. To distinguish between different cellular components, autophagy employs selective autophagy receptors, which will link the cargo to the autophagy machinery, thereby sequestering it in the autophagosome for its subsequent degradation in the lysosome. Autophagy receptors undergo post-translational and structural modifications to fulfil their role in autophagy, or upon executing their role, for their own degradation. We highlight the four most prominent protein modifications – phosphorylation, ubiquitination, acetylation and oligomerisation – that are essential for autophagy receptor recruitment, function and turnover. Understanding the regulation of selective autophagy receptors will provide deeper insights into the pathway and open up potential therapeutic avenues.

Introduction

Cells employ various pathways to maintain their homeostasis. Maintaining the balance between synthesis and removal of proteins, organelles or other cellular components is one of the major tasks of the cellular quality control mechanism. Accumulation of damaged or unwanted cellular components can increase the production of reactive oxygen species or DNA damage, and subsequently lead to cell death [1, 2]. To prevent this, cells have evolved two major pathways – the ubiquitin–proteasome system (UPS) and macroautophagy (hereafter autophagy), both of which are involved in the surveillance and quality control of proteins and organelles [3,4]. While UPS relies on tagging proteins with ubiquitin to target them for degradation within the barrel-shaped proteasome, autophagy allows the degradation of larger cellular components (including organelles) within the lysosome, mediated by a *de novo*-formed vesicle termed an autophagosome (reviewed in Ref. [3–7]). These pathways are finely regulated, and any disruptions to either of them can lead to neurodegeneration, cancer and other pathologies.

The term 'autophagy' was first used by Christian de Duve, in 1963 [8], before gaining significant traction

Abbreviations
AIM, ATG8-interacting motif; ATG, autophagy-related gene; cvt, cytoplasm-to-vacuole; ER, endoplasmic reticulum; GIM, GABARAP-interacting motif; LIR, LC3-interacting region; LLPS, liquid–liquid phase separation; PAS, pre-autophagosomal structure; PB1, Phox and Bem1; PI3K, phosphatidylinositol (3) kinase; PTMs, post-translational modifications; RHD, reticulon homology domain; SARs, selective autophagy receptors; UPS, ubiquitin–proteasome system.

upon the characterisation of autophagy-related proteins (Atg) in yeast [9,10], and has since been shown to have an essential role in higher eukaryotes. The core autophagy machinery is highly conserved from yeast to human, and it engages five multifunctional systems – (a) ULK1/ULK2 kinase complex (Atg1 in yeast); (b) the class III phosphatidylinositol 3-kinase (PI3K) complex; (c) the LC3/GABARAP proteins and their conjugation machinery (Atg8 in yeast); (d) autophagy-related gene 2 (ATG2; same in yeast) and phosphatidylinositol (e) phosphate effector proteins, including WIPI1–4 proteins (Atg18 and Atg21 in yeast); and (f) the membrane-spanning protein ATG9 (same in yeast; reviewed in Ref. [11,12]). The main event in autophagy is a *de novo* formation of a double membrane called phagophore, at endoplasmic reticulum (ER)-associated sites. The orchestrated action of the autophagy machinery components leads to the expansion of the phagophore, recruitment of the cargo and formation of an autophagosome. Autophagosomes fuse with lysosomes, resulting in the degradation of the cargo and recycling of the nutrients back into the cytosol [11]. Depending on the cargo, autophagy can be selective or nonselective. In nonselective autophagy, bulk portions of the cytoplasm are sequestered by the phagophore for degradation to maintain the nutrient levels in cells. This type of autophagy is frequently observed upon low nutrient levels during starvation. By contrast, selective autophagy is required for maintaining the number and integrity of cellular organelles, as well as protecting the cell from pathogen invasions. Selected cargo can be mitochondria, portions of ER, ribosomes, peroxisomes, lysosomes, lipid droplets, aggregates, ferritin and others [13,14]. The cargo is distinguished and selected by proteins called cargo receptors, or selective autophagy receptors (SARs), which act as a bridge between the phagophore and cargo (Table 1) [6,13–18]. Generally, cargo receptors are defined and characterised by their ability to bind cargo and facilitate the recruitment of autophagic machinery, mainly through the binding of Atg8/LC3/GABARAP proteins, and finally being degraded with the cargo [19]. The specific action of SARs is regulated by protein modifications, predominantly phosphorylation, ubiquitination, acetylation and oligomerisation, which are the focus of this review.

Table 1. Selective autophagy types and corresponding receptors.

Selective type of autophagy	Selected cargo	Species	SARs [references]
Aggrephagy	Aggregates	Yeast	Cue5 [25]
		Plants	NBR1 [112]
		Mammals	NBR1, OPTN, p62, TOLLIP, TAX1BP1 [96, 117–120]
BES1 degradation	BES1	Plants	DSK2A/B [69]
Cvt pathway	Ape1, Ape4, Ams1	Yeast	Atg19, Atg34 [22, 23]
ER-phagy	ER	Yeast	Atg39, Atg40 [26]
		Plants	ATI3, C53 [44, 121]
		Mammals	ATL3, C53, CCPG1, FAM134B, RTN3, Sec62, TEX264, CALCOCO1 [36, 44, 66, 103, 122–126]
Ferritinophagy	Ferritin	Mammals	NCOA4 [56, 127]
Glycophagy	Glycogen	Mammals	STBD1 [128]
Lipophagy	Lipid droplets	Mammals	p62 [129, 130]
Lysophagy	Lysosomes	Mammals	p62, TRIM16 [131, 132]
Midbody removal	Midbody rings	Mammals	NBR1, p62, TRIM16 [133–135]
Mitophagy	Mitochondria	Yeast	Atg32 [27]
		Mammals	AMBRA1, Bcl2L13, BNIP3, Cardiolipin, Ceramide, FKBP8, FUNDC1, NDP52, NIX, NLRX1, OPTN, p62, PHB2, TAX1BP1 [46, 79, 136–147]
NPC-phagy	Nuclear pore complex (NPC)	Yeast	Nup159 [148, 149]
Nucleophagy	Nucleus fragments	Yeast	Atg39 [26]
Pexophagy	Peroxisomes	Yeast	Atg30, Atg36 [28, 29]
		Mammals	NBR1, p62 [150, 151]
Plastid-to-vacuole	Plastids	Plants	ATI1 [152]
Proteaphagy	Proteasome	Plants	RPN10 [153]
Ribophagy	Ribosomes	Mammals	NUFIP1 [154]
Xenophagy	Bacteria and viruses	Mammals	NDP52, OPTN, p62, TAX1BP1 [70, 155–157]
		Plants	NBR1 [158]
Zymophagy	Zymogen granules	Mammals	p62 [159]

Selective autophagy receptors

The main steps in selective autophagy include recognition of the cargo, coupling of the cargo to the phagophore and degradation of the cargo. In particular, the first two steps depend on SARs – they recognise and bind the cargo, and facilitate formation of the growing phagophore [16].

The first type of selective autophagy was identified in *Saccharomyces cerevisiae*. The cytoplasm-to-vacuole pathway, commonly known as Cvt pathway, delivers the enzymes aminopeptidase 1, aspartyl aminopeptidase and α-mannosidase (Ape1, Ape4 and Ams1, respectively) into the vacuole [20,21]. The recognition of these enzymes is achieved by Atg19 and Atg34 receptors. Both Atg19 and Atg34 can specifically bind the cargo proteins, Atg11 – the scaffolding subunit of Atg1 complex – and Atg8 proteins, which decorate the pre-autophagosomal structure (PAS), thereby recruiting the cargo into the Cvt pathway [22–24]. Selective degradation of mitochondria, peroxisomes, ER, parts of nuclei and aggregates by autophagy has also been studied in yeast, with each of these degradation pathways employing autophagy receptors. Yeast SARs commonly bind Atg8 and Atg11, with the exception of Cue5, which has shown no interaction with Atg11 [25–32]. In yeast, binding to Atg8 proteins is mediated by a W/F/YxxL/I/V motif, commonly known as Atg8-interacting motif (AIM), characterised by hydrophobic residues on positions 0 and +3, and often surrounded by acidic residues to stabilise the interaction [33].

Higher eukaryotes come with additional layers of complexity to selective autophagy pathways, which have been extensively studied. In mammals, over 30 autophagy receptors have been identified and described (reviewed in Ref. [13,14]). Yeast Atg8 has six homologues in mammals, corresponding to LC3 and GABARAP families – LC3A, LC3B, LC3C and GABARAP, GABARAP L1 and GABARAP L2 (also known as GATE-16). Mammalian SARs bind LC3/GABARAP proteins through conserved AIM-like LC3-interaction region and GABARAP-interacting motif (LIRs and GIMs, respectively) [33–35]. The LIR/GIM region, similarly to AIM, is characterised by hydrophobic residues at positions 0 and +3 and frequently flanked by negatively charged amino acids that contribute to reinforcing the binding [34,35].

Interestingly, whereas the majority of yeast SARs interact with the scaffold protein Atg11, which recruits Atg1 complex to cargo, only some mammalian SARs interact with its functional mammalian homologue, FAK-family-interacting protein of 200 kDa (FIP200), which can drive the recruitment and activation of ULK1 kinase and the downstream machinery to the selected cargo, thereby facilitating phagophore formation [36–43]. In plants, selective autophagy has not been as extensively studied; however, a number of SARs have been reported to interact with ATG8 in an AIM-dependent manner [44,45].

In addition to ATG8/LC3/GABARAP-binding sites, SARs generally possess cargo-binding domains. These are often ubiquitin-binding domains, since autophagic cargo is frequently ubiquitinated. Ubiquitin-independent cargo recognition involves SARs binding directly to the cargo, as well as SARs recognising sugars and lipids exposed on the cargo (Fig. 1; [46–51], reviewed in Ref. [52]).

Regulation of autophagy receptors

For its full functionality and to minimise costly energy losses, autophagy flux is tightly regulated (reviewed in Ref. [53–55]). SARs undergo structural and post-translational modifications (PTMs) in order to operate in a specific type of autophagy, to facilitate binding to other proteins or simply to be removed via the proteasome. Below, we outlined the most prominent SAR modifications, such as phosphorylation, ubiquitination, acetylation and oligomerisation (Fig. 2; Table 2), and explain how they affect SARs within autophagy pathways.

Ubiquitination

Ubiquitination is commonly mentioned in the context of protein degradation, as it serves as a signal for protein removal by either the proteasome or autophagy [6]. SARs are also ubiquitinated for their own degradation. An example is NCOA4, a ferritinophagy receptor, involved in selective degradation of ferritin. Ferritin is a protein that serves to store iron in cells and release it in a controlled fashion when iron levels are low [56,57]. NCOA4 is an iron-sensing protein, which engages its C-terminal helical domain in coordinating iron. As such, during high-iron levels, NCOA4 interacts with and is ubiquitinated by HERC2 ubiquitin ligase, thereby preventing ferritin degradation and iron release [57]. Regulation of ferritinophagy is essential for maintaining iron levels in cells, and any perturbations within this process could lead to pathologies, such as anaemia, or neurodegeneration, such as neurodegeneration with brain iron accumulation (NBIA) [58].

Ubiquitination may not only signal for SAR degradation, but also regulate their function in autophagy. For example, p62, a SAR involved in the regulation of

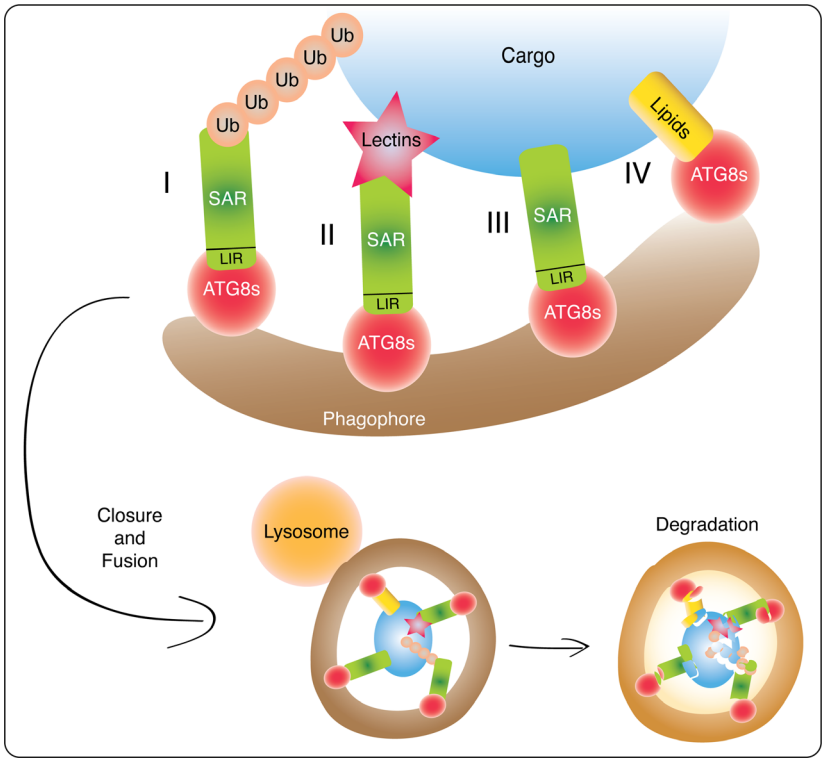

Fig. 1. Types of cargo recognition in selective autophagy. During selective autophagy, cargo is carefully selected in several ways for its degradation within the lysosome. (I) In ubiquitin-dependent autophagy, cargo is polyubiquitinated, which serves as a signal for its degradation. Cargo receptors can simultaneously bind ATG8 proteins and polyubiquitin on the cargo, providing a strong link between autophagy machinery and the cargo itself. (II) Cargo receptors can also bind sugars, such as lectins, that are recruited to the cargo, as is the case during lysophagy, where galectin 3 decorates damaged lysosomes and is able to bind the receptors. (III) SARs can also bind directly to the cargo and deliver it to the forming phagophore through the ATG8 binding. (IV) Some lipids (such as cardiolipin) can bind ATG8s and provide a direct link between cargo and autophagy machinery in a receptor-independent manner. Phagophore elongates, surrounding the cargo. When it seals, it fuses with the lysosome and the cargo is degraded.

redox homeostasis, undergoes nondegradative ubiquitination. During normal cellular conditions, Kelch-like ECH-associated protein 1 (KEAP1) interacts with nuclear factor erythroid 2-related factor 2 (NRF2), a transcription factor involved in gene expression of antioxidant proteins. Through its association with Cullin3 E3 ligase, KEAP1 mediates the ubiquitination of NRF2, targeting it for proteasomal degradation. Upon oxidative stress, it is the task of p62 to sequester KEAP1 within aggregates for autophagic degradation in order to release NRF2, which can then translocate to the nucleus and trigger an antioxidative response (reviewed in Ref. [59]). Pan and colleagues have reported that this process can be blocked by ubiquitination of p62 at Lys7 [60], which would normally form a hydrogen bond with Asp69, driving homodimerisation of p62 via its Phox and Bem1 (PB1) domain [61,62]. PB1-mediated homodimerisation is a prerequisite for its sequestering function. Ubiquitinated p62 can no longer mediate autophagic degradation of KEAP1, suppressing the release of NRF2 and stimulating antioxidative actions to restore redox homeostasis [60].

Conversely, SAR ubiquitination can also be a signal for autophagy activation and cargo recognition. KEAP1/Cullin3 also directly ubiquitinates p62 at Lys420, within its UBA domain. The ubiquitination prevents p62 dimer formation through the UBA domain, thereby enhancing its sequestration ability and interactions with phagophores and LC3/GABARAP proteins during ubiquitin stress [63,64]. A similar ubiquitination pattern has been observed for NBR1, with ubiquitin-stress-triggered ubiquitination likely regulating its interaction with polyubiquitin chains [63]. Furthermore, OPTN stably interacts with HACE1 E3 ubiquitin ligase, which leads to HACE1-mediated ubiquitination of OPTN at Lys193, facilitating the complex formation between OPTN, p62 and

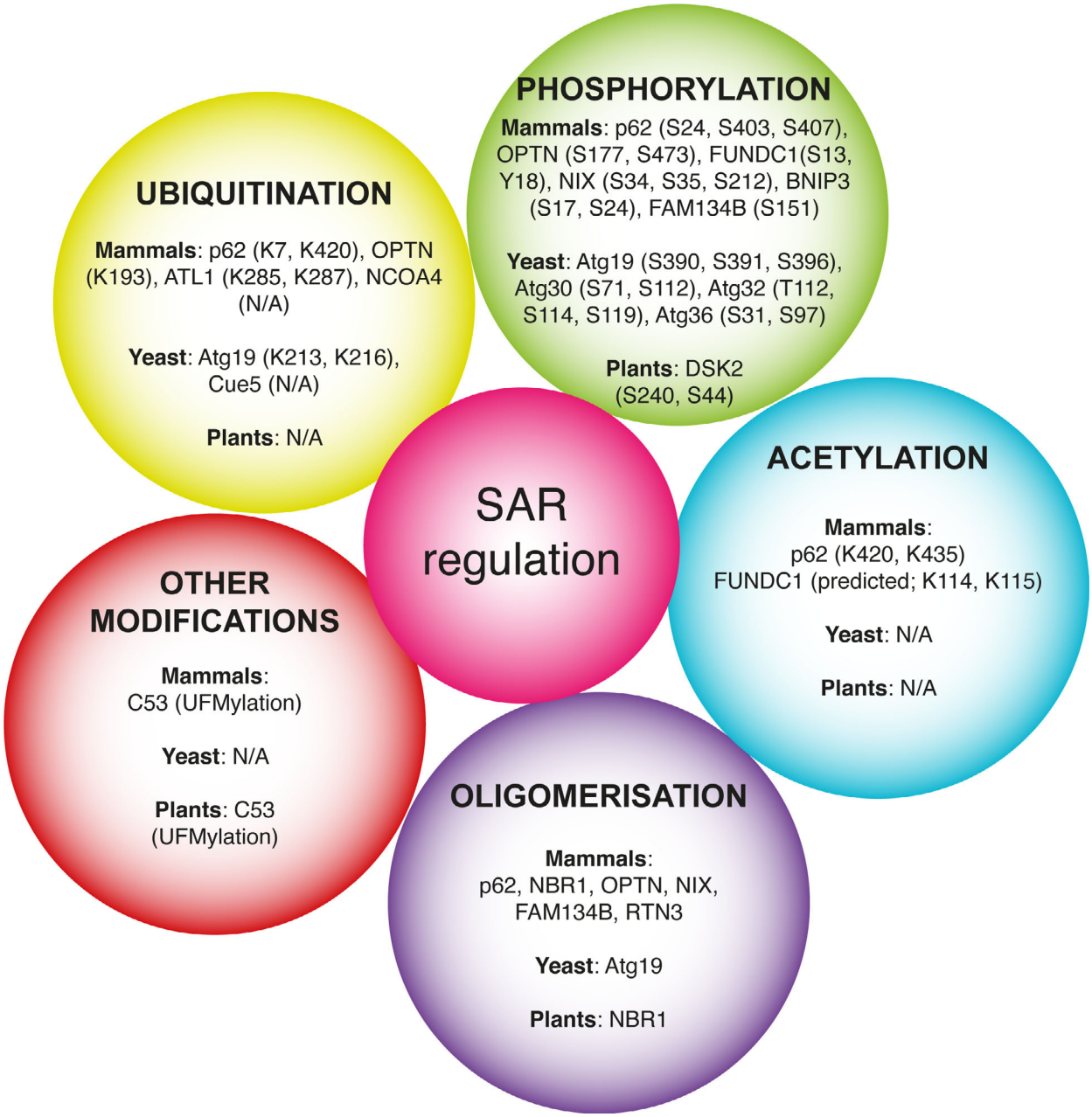

Fig. 2. Summary of regulatory modifications reported for SARs. SARs are regulated through post-translational, structural and other modifications, most prominent ones summarised in this scheme. SARs and their modified sites are listed under the regulation modification that affects them.

HACE1, as well as to coupling of cargo to the autophagic machinery. HACE1 is downregulated in various cancers, suggesting that the HACE1-OPTN axis may play a role in tumour suppression [65].

There is also preliminary evidence for functional regulation of ER-phagy receptors, but not much is known about ubiquitination of ER-phagy SARs. Atlastins 1 and 3 (ATL1 and ATL3), membrane-bound dynamin-like GTPases, have been found to function as ER-phagy receptors, binding GABARAPs and targeting parts of the tubular ER for autophagy [66]. Atlastins are involved in the fusion of ER tubules, which is regulated through ubiquitination by SYVN1 E3 ligase [67].

Table 2. Autophagy-associated SAR modifications with corresponding enzymes.

Protein	Species	Residues (enzymes, if known)	References
Phosphorylation			
p62	Mammals	S24 (PKA), S403 (CK2, ULK1, TBK1), S407 (ULK1)	[80–82, 160]
OPTN	Mammals	S177, S473 (both TBK1)	[50, 70]
FUNDC1	Mammals	S13 (CK2/phosphatase PGAM5), Y18 (SRC)	[72, 73]
NIX	Mammals	S34, S35, S212	[71, 102]
BNIP3	Mammals	S17, S24	[161]
FAM134B	Mammals	S151 (CAMK2B)	[83]
Atg19	Yeast	S390, S391, S396 (Hrr25)	[75, 77]
Atg30	Yeast	S71, S112	[31]
Atg32	Yeast	T112, S114 (CK2), S119 (CK2)	[31, 74]
Atg36	Yeast	S31 (N/A), S97 (Hrr25)	[31, 77]
DSK2	Plants	S240, S244 (+more) (BIN2)	[69]
Ubiquitination			
p62	Mammals	K7 (TRIM21), K420 (Cullin3)	[60, 63, 64]
OPTN	Mammals	K193 (HACE1)	[65]
ATL1	Mammals	K285, K287 (SYVN1)	[67]
NCOA4	Mammals	N/A (HERC2)	[57]
Atg19	Yeast	K213, K216 (Ubp3p)	[68]
Cue5	Yeast	N/A (Rsp5)	[25]
Acetylation			
p62	Mammals	K420, K435	[86]
FUNDC1	Mammals	K114, K115 (predicted)	[89]
Oligomerisation			
p62	Mammals	K7, R21, D69, E82, E409, G410	[62, 99]
NBR1	Mammals		[96]
OPTN	Mammals	E50	[43, 162]
NIX	Mammals	G204, G208	[102]
FAM134B	Mammals	G216	[83]
RTN3	Mammals		[103]
Atg19	Yeast		[98]
NBR1	Plants	K11, K19, D60, D73	[112]
UFMylation			
C53	Mammals Plants	N/A (UFL1)	[44]

In yeast, not many examples of ubiquitination events regulating cargo receptor function have been reported. However, Baxter and colleagues have shown that Atg19, the Cvt pathway receptor, interacts with and is ubiquitinated by the E3 ligase Ubp3 at lysines 213 and 216. Downregulation of this ubiquitination results in decreased Cvt targeting [68].

Ubiquitination is one of the most abundant PTMs in cells, and it is likely that many more SARs are regulated in this way. Dysregulation of autophagy can have pathological consequences, and thus, understanding the regulation of SARs by ubiquitination may provide the basis for therapeutic approaches.

Phosphorylation

Phosphorylation plays an essential part in regulating autophagic flux and is central to autophagy induction. Moreover, it plays an important role in regulating the ability of SARs to bridge the cargo with autophagic machinery. In most cases, phosphorylation regulates SARs binding to Atg8/LC3/GABARAP [31,69–73]. This has been shown already in yeast, and is conserved across species, including humans. In yeast, pexophagy receptors Atg30, Atg32 and Atg36 interact with Atg8 in an AIM-dependent manner. These interactions are facilitated by phosphorylation at residues upstream of the respective AIM regions [31]. Furthermore, Hrr25 and casein kinase 2 (CK2) have been shown to phosphorylate yeast SARs and facilitate their interaction with the scaffold protein Atg11 [74–78].

Plant autophagy SARs are also modified by phosphorylation. During stress conditions, such as drought and starvation, it is required to strengthen the interaction of SARs, such as DSK2, with ATG8 [69].

In mammals, TBK1 phosphorylates OPTN at Ser177 during xenophagy, which strengthens its interaction with LC3s/GABARAPs and subsequently restricts *Salmonella* growth [70], and at Ser473, which facilitates its interaction with ubiquitin chains during mitophagy [79]. Similarly, phosphorylation of Nix at Ser34 and Ser35 enhances its interaction with LC3s and initiates mitophagy [71]. Conversely, phosphorylation of FUNDC1 at Tyr18 prevents binding to LC3s/GABARAPs, thereby inhibiting mitophagy [72]. During hypoxia, the interaction of FUNDC1 with LC3/GABARAP proteins is determined by the phosphorylation of Ser13, which is regulated by the interplay of a phosphoglycerate mutase (PGAM5) phosphatase and casein kinase 2 [73].

p62 function in autophagy is also regulated by various kinases. During proteotoxic stress, the phosphorylation at Ser407 by ULK1 kinase allows the transition of p62 from dimer to a monomer, promoting its binding to ubiquitin, and allowing its recruitment to protein aggregates [80]. Similarly, phosphorylation at Ser403 by various kinases enhances the binding to ubiquitin chains [80–82], subsequently promoting cargo degradation.

Phosphorylation also positively regulates FAM134B-dependent ER-phagy. CAMK2B kinase phosphorylates FAM134B at Ser151 within its reticulon homology domain (RHD) in order to facilitate

FAM134B dimerisation and membrane fragmentation, thereby making it accessible for autophagosome sequestration [83]. Many other SARs undergo phosphorylation modifications as part of their regulation mechanism, with further studies needed to understand them and identify new ones [79,84,85].

Acetylation

Although equally essential, it took some time for acetylation to attract attention of autophagy researchers, with new roles now rapidly emerging. Acetylation involves the addition of an acetyl group onto lysines or amino-termini of proteins. During selective autophagy, UBA domain-mediated oligomerisation of p62 and its interaction with polyubiquitinated cargo are regulated by acetylation. The activation of acetyltransferase TIP60 drives p62 acetylation at Lys420 and Lys435, facilitating the binding to polyubiquitinated proteins [86]. Ser403 and Ser407 phosphorylation, found in close proximity to acetylated lysines, regulates a similar function of p62 [80,81]. It is likely that there is a link between the two PTMs, although further research is needed to demonstrate this.

LC3 proteins, which shuttle between the nucleus and the cytosol, are also regulated by acetylation. Upon starvation, LC3 is deacetylated by Sirt1 at lysines 49 and 51, driving its translocation from the nucleus into the cytosol, where it is able to participate in the process of autophagy [87].

Acetylation is implicated in regulating ER-phagy SARs through the modification of ATG9A protein. ATG9A interacts with FAM134B and Sec62, two ER-phagy receptors. This interaction is reduced upon acetylation of ATG9A, which prevents binding of ER-phagy receptors to LC3s and inhibits ER-phagy induction. This can be enforced in transgenic mice that overexpress ATase1 (AT-1) in forebrain neurons, causing a progeria-like phenotype. AT-1 is required for translocating cytosolic acetyl-CoA into the ER lumen [88].

FUNDC1 is also possibly regulated by acetylation, as potential acetylation sites on it have been predicted [89], but more work is required to understand its effect and relevance. Acetylation offers an interesting approach in terms of potential therapeutic targets, as exemplified by AT-1 and ER-phagy. However, our knowledge of this modification in autophagy is still at the very beginning.

Oligomerisation

Protein function, structure or localisation is not only regulated by PTMs. Oligomerisation has been shown to have an important part in regulating the induction of autophagy, either by promoting kinase activation, such as oligomerisation of ULK1 [90], or by providing the structural architecture for protein scaffold formation on the phagophore, with class III PI3K complex and ATG16L1 as good examples [91,92].

In yeast, it has recently been shown that oligomerisation of Atg17 is prerequisite for liquid–liquid phase separation (LLPS) of the PAS [93,94]. Moreover, LLPS has been shown to regulate the lysosomal degradation of Ede-1-dependent endocytic protein deposits, which are formed as a consequence of defects in early stages of clathrin-mediated endocytosis. In this case, Ede-1 has a role of cargo receptor, and its polymerisation drives LLPS. Through its binding to Atg8 and Atg11, Ede-1 drives the degradation of the formed condensates [95].

Many SARs contain a region in their sequence that allows oligomerisation. Autophagic cargo is generally larger than that of the proteasome, as it mainly consists of organelles or macromolecules, which can attribute to the necessity of autophagy receptors to oligomerise. Furthermore, oligomerisation of SARs allows better exposure of their domains and moieties, as well as increases their binding avidity. To better fulfil their role as bridges between autophagic machinery and cargo, many SARs homo- and hetero-oligomerise, as shown for p62 and NBR1 [96,97]. Additionally, autophagic cargo itself is known to oligomerise, to allow efficient autophagic uptake, as it is the case with ferritin during ferritinophagy [56].

During the yeast Cvt pathway, the cargo proteins Ape1 and Ams1 form dodecamers and tetramers, respectively, allowing efficient cargo packaging. Ape1, as more abundant cargo, carries Ams1 to facilitate its binding to Atg19, which forms a trimer. Atg19 trimers are incorporated into the Ape1/Ams1 aggregates, thereby controlling the size of the assembled cargo. These large aggregates will be efficiently engulfed by the Cvt vesicle and delivered to the vacuole [98].

In mammals, oligomerisation has been extensively studied in the context of SAR regulation. The effect of PTMs on p62 has frequently been attributed to the regulation of its ability to oligomerise. p62 can oligomerise through its PB1 domain and through its UBA domain, which has different consequences for autophagy [62,99]. p62 polymerisation has been found to be one of the requirements of p62 to undergo phase separation, along with ubiquitin binding through its UBA domain, which drives the concentration and sequestration of the autophagic cargo [49].

It has been shown that OPTN forms homo-hexamers, with its role currently unknown [100]. However,

OPTN E50K mutation, associated with normal tension glaucoma (NTG), has been shown to inhibit oligomerisation and solubility of OPTN, and at the same time strongly enhance its interaction with TBK1 [101].

Recently, Nix has been shown to dimerise, thereby stabilising its interaction with LC3A and its function as a cargo receptor. This dimerisation is regulated by phosphorylation at Ser212 [102].

ER-phagy cargo receptors commonly undergo oligomerisation as part of their regulation mechanism. FAM134B oligomerisation is facilitated by the phosphorylation of its RHD by CAMK2B kinase, regulating ER membrane scission for ER-phagy demands [83]. RTN3L, a long isoform of RTN3 reticulon, can homodimerise and heterodimerise with RTN3S isoform, and both have different functions during ER-phagy [103]. RTN3L homodimers can drive fragmentation of ER tubules, which will be subsequently delivered to the lysosome for degradation [103]. Conversely, RTN3L-RTN3S heterodimers contribute to stabilisation of ER tubules instead [103]. In *Drosophila*, Atlastin has also shown a tendency to oligomerise, in GTP-dependent manner. The formation of this homo-oligomer is required for ER membrane tethering and fusion [104,105], with similar role observed with mammalian Atlastins ATL1, ATL2 and ATL3 [106–111]. However, it remains to be discovered how oligomerisation affects ATL1 and ATL2 receptor functions in mammals.

Plant SARs also rely on oligomerisation for their function. Plant NBR1, a functional hybrid of mammalian NBR1 and p62, undergoes oligomerisation via its PB1 domain, similarly to p62, which is required for its role in autophagy [112]. Oligomerisation has also been observed in other SARs, with its function yet to be completely understood [57,113,114], but likely involved in facilitating the linkage of SARs with the cargo and autophagic machinery.

Concluding remarks

With various cell types and different organelles, macromolecules, aggregates and pathogens all targeted for degradation by autophagy, across species, cells have developed a large number of SARs, and evolved tight regulatory mechanisms, since most of the SARs also have additional functions to being cargo receptors. PTMs such as phosphorylation, ubiquitination and acetylation have been extensively studied (Fig. 2; Table 2); hence, much is known about their role in regulating SARs. In this review, we summarised the most prominent and best characterised modifications of SARs and how they affect the function of SARs in their respective pathways. Along with PTMs, translational (reviewed in Ref. [115]) and structural changes can be involved in regulating the SAR function. The orchestrated action of multiple PTMs is a rapidly emerging theme in autophagy and beyond. Such crosstalk of PTMs provides an additional layer of complexity and fine-tuning of regulatory mechanisms. Phosphorylation is frequently reported to regulate SAR binding to ubiquitin chains of the targeted cargo [50,80,81], but it can also regulate ubiquitination of the protein itself. PTMs can regulate the same function of one SAR, as shown with p62, with acetylation and phosphorylation, in addition to oligomerisation, all regulating its binding to ubiquitinated cargo [80,81,86]. Conversely, same cargo receptors can be regulated by several different modifications, all of which will regulate different parts of their function, again with p62 being best characterised example [43,63,80–82,85,86,97].

The question of additional PTMs in relation to SARs remains largely unexplored part of the field, but processes such as methylation, glycosylation, SUMOylation and ISGylation could have an important role in SAR regulation. UFMylation has recently emerged as an interesting process involved in regulating ER-phagy [44,116] (Fig. 2; Table 2), with ER-phagy receptor C53 being the only ER-phagy cargo receptor known to be regulated by UFMylation thus far. This regulation has been shown in plants and is conserved in mammals [44]. The rise of UFMylation within the field provides novel insights into the regulatory mechanism of ER-phagy.

And while particular modifications have been identified for many SARs, the identity of enzymes catalysing said modifications is not always clear. Therefore, it is important to understand the identity of kinases, phosphatases, E3 ligases, deubiquitinases, acetylases, deacetylases and others, especially in the context of pathologies. Since any disorder in the process of autophagy, as well as SARs, has been linked to changes in immune response and, therefore pathologies, such as infection, neurodegeneration and cancer, further research of the regulatory mechanisms of SARs will shed light and open new avenues of therapeutic strategies.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

AG and ID wrote and revised the manuscript and designed the figures.

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