Selective Autophagy: ATG8 Family Proteins, LIR Motifs and Cargo Receptors

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Abstract

Selective autophagy relies on soluble or membrane-bound cargo receptors that recognize cargo and bring about autophagosome formation at the cargo. The cargo-bound receptors interact with lipidated ATG8 family proteins anchored in the membrane at the concave side of the forming autophagosome. The interaction is mediated by 15- to 20-amino-acid-long sequence motifs called LC3-interacting region (LIR) motifs that bind to the LIR docking site (LDS) of ATG8 proteins. In this review, we focus on LIR–ATG8 interactions and the soluble mammalian selective autophagy receptors. We discuss the roles of ATG8 family proteins as membrane scaffolds in autophagy and the LIR–LDS interaction and how specificity for binding to GABARAP or LC3 subfamily proteins is achieved. We also discuss atypical LIR–LDS interactions and a novel LIR-independent interaction. Recently, it has become clear that several of the soluble cargo receptors are able to recruit components of the core autophagy apparatus to aid in assembling autophagosome formation at the site of cargo sequestration. A model on phagophore recruitment and expansion on a selective autophagy receptor-coated cargo incorporating the latest findings is presented.

Introduction

The term autophagy describes a set of lysosomal degradation pathways of cytoplasmic components ranging from single proteins to large organelles, like mitochondria and parts of the endoplasmic reticulum (ER) [1]. Although there are several variations over the theme, we usually define the different pathways as macroautophagy, microautophagy and chaperone-mediated autophagy. Chaperone-mediated autophagy is in principle a selective process where single polypeptides are recognized as unfolded/misfolded when exposing binding sites (degenerate KFERQ-like pentapeptide motifs) for heat shock-cognate protein of 70 kDa (Hsc70) [2]. Microautophagy can be both unselective and selective and involves the direct uptake of cytoplasmic material by lysosomes or endosomes, either by invaginations or by protrusions of their limiting membrane [3,4]. In this review, we focus on the role of cargo receptors and LC3 interaction region (LIR) motifs in selective macroautophagy. For the sake of simplicity, we will in the following refer to macroautophagy as autophagy.

The core machinery required for autophagosome formation is largely conserved from yeast to man [5–8]. In vertebrates, the core autophagy components can be grouped into five functional complexes including the ULK protein kinase complex with ULK1 and -2, ATG13, ATG101 and FIP200 (also called RB1CC1); the class III phosphoinositide 3-kinase (PI3K) complex I, with VPS34, VPS15, Beclin 1 and ATG14; the phosphatidylinositol-3-phosphate (PI3P)-binding ATG2A or -B and WIPI1–4 complex; and the two ubiquitin-like (UBL) conjugation systems with ATG5–ATG12 and mammalian ATG8 proteins conjugated to phosphatidylethanolamine (PE) with ATG7 acting as E1 in both conjugation pathways, ATG10 as E2 in the ATG5–ATG12 pathway and ATG3 as E2 in the ATG8–PE pathway where the ATG5–ATG12:ATG16L1 complex acts as an E3 ligase. The four ATG4A–D cysteine proteases, with ATG4B as the dominant acting one, process the

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Table 1. Mammalian selective autophagy receptors

Pathway	*Substrate*	*Mammalian autophagy receptors*	*Refs*
Aggrephagy	Protein aggregate	p62, NBR1, OPTN	[17,18,126,175]
Ub-dependent Mitophagy	Mitochondria	NDP52, OPTN, p62, TAX1BP1, AMBRA1	[142,143,176–178]
Ub-independent Mitophagy	Mitochondria	NIX, BNIP3, FUNDC1, Bcl2L13, FKBP8, PHB2, NLRX1, AMBRA1, cardiolipin, ceramide	[58,73,101,102,177,179–183]
Ub-dependent Pexophagy	Peroxisome	NBR1, p62	[139,184]
Lysophagy	Lysosome	TRIM16, NDP52	[116,152]
Zymophagy	Secretory granule	p62	[185]
ERphagy	ER	FAM134B, SEC62, RTN3, CCPG1, ATL3, TEX264	[172,186–191]
Ferritinophagy	Ferritin	NCO4A	[105, 106]
Glycophagy	Glycogen	Stbd1	[107]
Nuclear lamina autophagy	Nuclear lamina	Lamin B1	[103]
Xenophagy	Bacteria	NDP52, p62, OPTN, TAX1BP1	[80,90,144,145]
Virophagy	Viral capsids	TRIM5α, p62	[82,192]
Ribophagy	Ribosomes	NUFIP1	[108]
Midbody autophagy	Midbody rings	p62, NBR1, TRIM17	[153,193,194]

precursors of ATG8 proteins so they can become lipidated and also delipidates them. Finally, ATG9 is the only integral membrane protein essential for autophagy.

In yeast, the autophagosomes are born at a single site close to the vacuole (the yeast lysosome) called the phagophore assembly site (PAS) where the complexes of the core autophagy apparatus congregate [9]. In mammals, autophagosomes are generated at multiple sites and the membrane sources for phagophore generation are also several, including ER as the likely main source, with Golgi, recycling endosomes and the

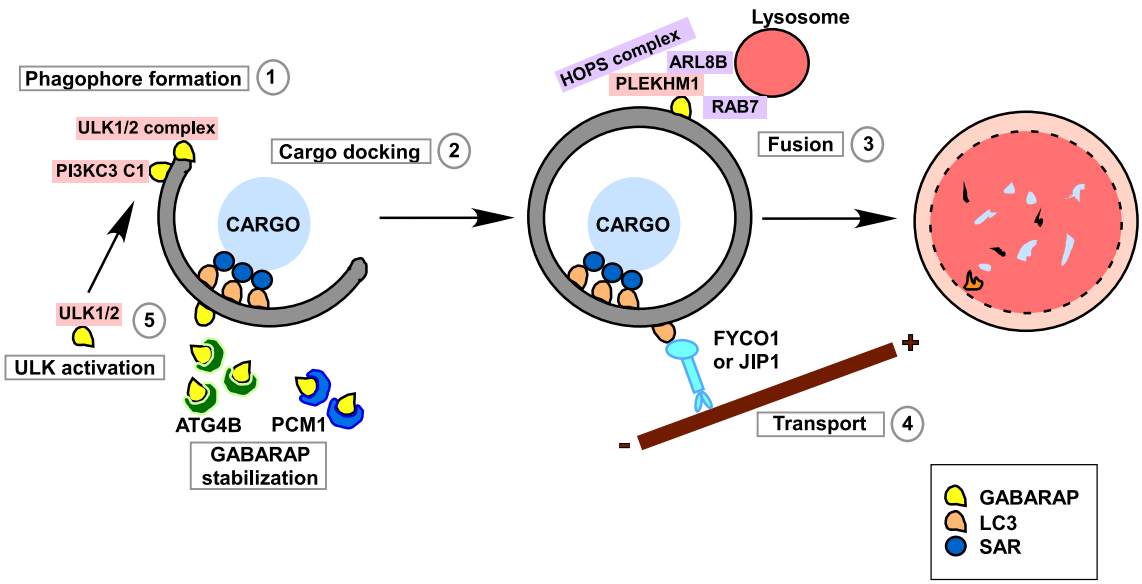

Fig. 1. Main functions of ATG8–LIR interactions in autophagy. GABARAP lipidated to the rim and outer surface of the phagophore facilitates phagophore growth by recruiting core autophagy proteins via LIR motifs (1). The LIR-dependent interaction of ATG8s like LC3B or LC3C, lipidated to the inner surface of the phagophore, to SARs is essential for the docking of cargo to the growing phagophore (2). LC3B or GABARAP lipidated to the closed autophagosome facilitates autophagosome fusion (3) or transport (4), respectively, by recruiting essential proteins like PLEKHM1 (3) or FYCO1, JIP1 (4) via LIR motifs. LIR interactions with unlipidated ATG8s may also play a regulatory role. ATG4 proteases uses a LIR to interact with the unprocessed ATG8 proforms, and after processing unlipidated GABARAP and GABARAPL1 are stabilized by their LIR interaction with ATG4B (5). The centriolar satellite protein PCM1 also stabilizes a pool of unlipidated GABARAP. The LIR interaction of unlipdated GABARAP with ULK1 is essential for activation of ULK1 in response to starvation (5).

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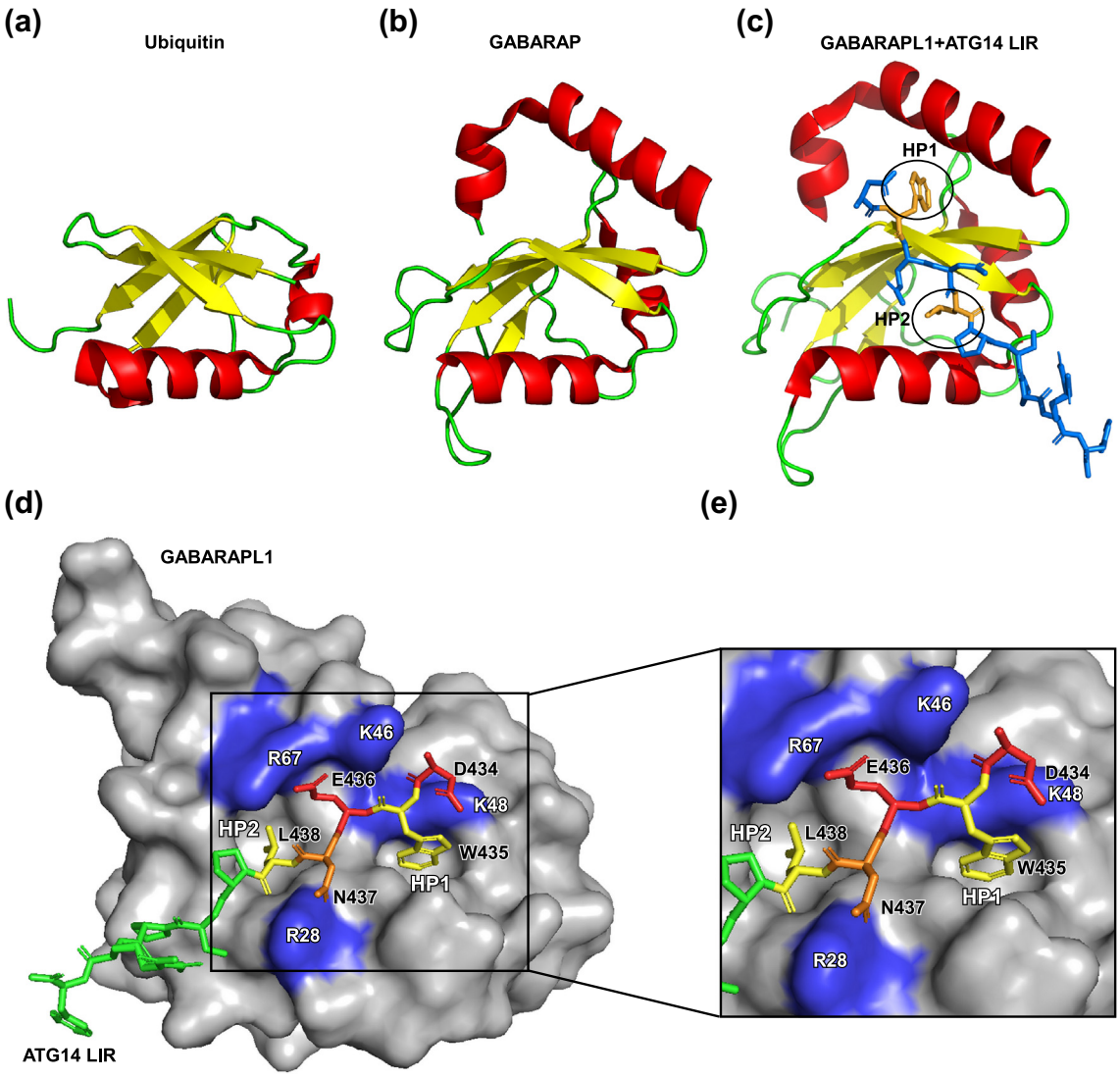

Fig. 2. Structures of ubiquitin (PDB: 2MVS; A), GABARAP (PDB: 1GNU; B) and the complex of GABARAPL1 with the ATG14 LIR peptide (PDB: 6HOL; C–E). In panels A–C, α-helices are red; β-sheets, yellow; and loops, green. The side chain of the aromatic W435 residue dives into the HP1 (hydrophobic pocket 1) and the aliphatic side chain of L438 is buried in HP2. Basic residues in the LDS of GBARAPL1 are colored blue. D434 and E436 engage in electrostatic interactions with K48 and R67, respectively. Figures were prepared using PYMOL (http://www.pymol.org).

plasma membrane as other sources [10]. The ULK1 complex with formation of FIP200- and ATG13-positive punctae can be considered a mammalian equivalent of PAS, mPAS [5]. It was recently shown that Syntaxin 17 (Stx17) phosphorylated on a certain Ser residue (S202) by Tank-binding kinase 1 (TBK1) translocates from Golgi to mPAS and controls formation of the FIP200- and ATG13-positive mPAS structures upon starvation in mammalian cells [11].

Although suggested earlier by de Duve (see review by Kirkin in this issue), the first strong evidence that autophagy acted selectively came from studies on the degradation of peroxisomes in yeast [12] and mitochondria in mice [13,14]. The concept of selective autophagy receptors (SARs) acting as cargo receptors was importantly fueled by the discovery of Atg19 acting as a selective receptor for targeting aminopeptidase I to the vacuole in the yeast biosynthetic cytoplasm to vacuole (Cvt) pathway [15,16]. The discovery of human p62/SQSTM1 (sequestosome-1) as the first bona fide SAR [17,18] initiated a race to discover mammalian SARs resulting in the identification of two broad groups of SARs: the soluble and the membrane-bound cargo receptors (see Table 1). SARs use their LIR motifs to interact with ATG8 family proteins attached to the inner membrane surface of the phagophore to ensure encapsulation of the cargo [19,20] (Fig. 1). In yeast and plants, the term ATG8-interacting motif (AIM) is also often used. In this

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review, we focus on the soluble mammalian SARs and LIR–ATG8 interactions. Before discussing the soluble SARs further, we will first consider the ATG8 family proteins and the LIR motif interactions.

ATG8 Family Proteins: Membrane Scaffolds and Tickets to the Lysosome

ATG8 proteins belong to a larger group of small UBL modifiers. They share the UBL fold. However, compared to ubiquitin, ATG8 proteins have two extra N-terminal α-helices (Fig. 2A and B). ATG8 proteins are expressed as cytosolic precursors that become processed at a C-terminal Gly residue by ATG4 proteins and conjugated to PE by the two conjugations systems involving ATG7, ATG10, ATG3, ATG5, ATG12 and ATG16 [5]. The lipidated (PE-conjugated) ATG8 proteins are anchored in the inner and outer membranes of the double membrane of the phagophore. The lipidation is reversible as ATG4 proteins can release the ATG8s from the membranes by cutting off the PE at the C-terminal Gly residue [21].

The lipidated ATG8 proteins act as adaptors or scaffolds for recruitment of LIR-containing proteins to both surfaces of the growing phagophore [22]. This way, they serve four main functions in autophagy (Fig. 1). One is membrane scaffolding of LIR-containing core autophagy components including ULK1, -2, ATG13 and FIP200 in the ULK1/2 complex [23]; VPS34, ATG14 and Beclin 1 in the class III PI3K complex I [24]; the ATG4B protease processing ATG8s [25]; and ATG12–ATG5 [26] to the rim and outer (convex) surface of the phagophore. Also in yeast, several of the core autophagy proteins are known to bind to Atg8 via LIR (AIM) motifs [27,28]. The main purpose of the interactions is presumably to scaffold and stabilize the binding of core autophagy proteins to the growing phagophore. The recently reported GABARAP interaction with ATG2A and -B is required for efficient phagophore closure [29]. A second function of lipidated ATG8 proteins is the LIR-dependent attachment of SARs (with our without cargo) to the inner (concave) surface of the phagophore [19,20]. Third and fourth, as discussed below, ATG8 proteins have also important roles in fusion of autophagosomes with lysosomes and in the transport of autophagosomes (for a recent review, see Ref. [30]).

In yeast, the single ATG8 protein is essential for phagophore expansion [31]. During the emergence of multicellular animals, two ATG8 subfamilies arose, the MAP1LC3 (microtubule associated protein 1 light chain 3) and GABARAP (GABA type A receptor-associated protein) subfamilies [32,33]. MAP1LC3 is usually referred to as LC3. The human ATG8 family proteins are encoded by seven genes expressing LC3A (two alternatively spliced isoforms with different N termini), LC3B, LC3B2 (only one amino acid differs from LC3B), LC3C, GABARAP, GABARAPL1 (GABARAP like 1) and GABARAPL2 [32]. Evolutionary analyses show that while LC3A–LC3B and GABARAP–GABARAPL1 originated from the same node, LC3C and GABARAPL2 (also called GATE-16) are in separate clades [33]. Some animal lineages have subsequently lost members so that i.e. *Caenorhabditis elegans* and *Drosophila* have only two ATG8s. Careful imaging studies performed by Mizushima's group revealed that autophagosomes do form and can fuse with lysosomes in cells knockout (KO) for the conjugation machinery (ATG7-, ATG5- and ATG3 KO cells). However, the flux is severely suppressed for both selective and nonselective autophagy in ATG conjugation-deficient cells that cannot lipidate ATG8s [34].

GABARAP and LC3 subfamilies: redundancy or specialization?

The evolutionary expansion of ATG8 proteins from yeast to man raises the question of redundancy *versus* functional specialization and diversification of mammalian ATG8 proteins. Nguyen *et al*. [35] used CRIPSR/CAS9 to KO all ATG8 family proteins in human HeLa cells. Interestingly, this study showed that ATG8 proteins are not essential for autophagosome formation but essential for autophagosome–lysosome fusion. KO of ATG8s resulted in smaller autophagosomes and a slowed initial rate of autophagosome formation. Also, the kinetics of autophagosome maturation are severely affected making the entire process very inefficient without functional ATG8 proteins [35]. Rescue experiments showed the GABARAP subfamily members to be more efficient in rescuing mitophagy and starvation-induced autophagy than LC3 subfamily members. A similar KO study from Wade Harper's group underscored the vital roles for GABARAP subfamily members in selective autophagy and found that LC3 subfamily members were not able to support all steps in selective autophagy [36]. Previously, siRNA-mediated knockdown experiments of human ATG8 family members performed by Engedahl's group revealed that autophagic bulk sequestration of cytosolic cargo is independent of LC3 but requires GABARAPs [37]. The dominant role of GABARAP subfamily proteins is consistent with yeast Atg8 being most homologous to human GABARAP and GABARAPL1. Also, the *C. elegans* GABARAP homolog, LGG-1, is required for autophagosome formation whereas loss of the LC3 homolog, LGG-2, only leads to formation of smaller autophagosomes [38]. It has also been reported that LGG-1 acts upstream of LGG-2, which is interacting with the HOPS complex to facilitate fusion with the lysosome [39]. An early study from Elazar's group, based on

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siRNA-mediated knockdown of ATG8 family members in HeLa cells, concluded that while both LC3 and GABARAP subfamily proteins are indispensable for efficient autophagy, LC3s are involved in elongation of the phagophore membrane, whereas the GABARAP subfamily is essential for a later stage in autophagosome maturation [40].

Early and late roles of GABARAPs

The central role of GABARAP proteins is also underscored by the fact that the LIR-containing core autophagy components, ULK1, -2, ATG13, FIP200 [23], VPS34, ATG14, Beclin 1 [24], ATG2A and -B [29], and ATG4B [25], bind preferentially to GABARAP. A recent study using knock-in mutations of the LIR motif of ULK1 and reconstitution of ATG13 KO cells with WT and LIR mutant constructs show that binding of GABARAP to both ULK1 and ATG13 is important for the activity of ULK1 and autophagosome formation. These interactions likely play a cooperative role to achieve optimal activation of ULK1 [41].

The KO studies mentioned above show that lack of GABARAP subfamily proteins leads to serious defects in autophagosome–lysosome fusion [35,36]. GABARAPs recruit the phosphatidylinositol-4-kinase PI4KIIα to autophagosomes, and PI4P generation on autophagosomes is critically important for fusion with lysosomes [42]. It is not known if this recruitment is LIR dependent. Fusion of the outer membrane of the autophagosome with lysosomes requires Rab GTPases, tethering factors, soluble N-ethylmaleimide-sensitive-factor attachment receptors (SNAREs) and other auxiliary proteins. Tethering factors bring together the membranes that are to fuse, bind to SNAREs and promote fusion. SNAREs connect the membranes that are to fuse by assembling into four α-helix bundles. The large multidomain scaffold protein and RAB7 effector pleckstrin homology domain containing protein family member 1 (PLEKHM1) interacts with the homotypic fusion and protein sorting (HOPS) tethering complex to mediate autophagosome–lysosome fusion. PLEKHM1 binds preferentially to GABARAP subfamily proteins in a LIR-dependent manner [43,44] (Fig. 1). PLEKHM1 also binds to the two GTPases RAB7 and ARL8B to mediate fusion [43,45].

Another RAB7 effector is EGP5, which binds to LC3 via two LIR motifs and also interacts with assembled STX17–SNAP29 to stabilize and assemble trans-SNARE proteins to facilitate autophagosome–lysosome fusion [46]. The SNARE syntaxin-17 (STX17) is acting both early in autophagosome formation and in autophagosome–lysosome fusion [11,47]. STX17 binds to both ATG8s and the small GTPase IRGM, which also binds to ATG8s. The resulting complex is efficiently translocated to autophagosomes [48]. STX17 binds to ATG8s through a LIR motif within the SNARE domain, while IRGM binds to ATG8s via a LIR-independent mode independent of the LDS [48]. Acting late in the fusion between autophagosomes and lysosomes, STX17 recruits the cytosolic SNARE SNAP-29. The complex is stabilized by homo-oligomeric ATG14 acting as a tether that also primes the STX17–SNAP29 binary t-SNARE complex on autophagosomes for VAMP8 interaction to promote autophagosome–endolysosome fusion [49]. ATG14 was recently shown to bind to GABARAPs via a LIR motif close to its C-terminal, membrane binding amphipathic α helix domain [24]. This interaction is important early during autophagosome formation, but may perhaps also play a role at the fusion step too. After catalyzing fusion, SNAREs are disassembled by *N*-ethylmaleimide-sensitive factor (NSF) acting together with the cofactor alpha-soluble NSF attachment protein (α-SNAP). GABARAP is known to bind to NSF [50], and may perhaps recruit NSF and α-SNAP to autophagosomes to mediate the SNARE disassembly after fusion.

Regulatory role of unlipidated GABARAP

Studies from the group of Sharon Tooze show that GABARAP is associated with ULK1 independent of lipidation and nutrient starvation. The two Golgi proteins WAC and GM130 bind to the unlipidated pool of GABARAP and regulate its subcellular localization. GM130 inhibits autophagy by tethering GABARAP to the Golgi. WAC counteracts this inhibitory interaction during starvation allowing GABARAP to translocate to the centrosome [51]. The archetypal centriolar satellite protein PCM1 recruits GABARAP, not LC3B, to the centrosome via a LIR motif that preferentially binds to GABARAP [52,53]. Upon starvation, GABARAP is then transported to mPAS. Activation of ULK1 by GABARAP requires the LIR motif. Knockdown of GABARAP specifically attenuates ULK1 activation [51]. PCM1 binds to the centriolar satellite E3 ligase MIB1 and can this way also regulate GABARAP stability [51,52]. Taken together, this work suggests that unlipidated GABARAP regulates phagophore expansion by activating the ULK complex. There are also pools of unlipidated GABARAP and GABARAPL1 bound to ATG4B in the cell [25] (Fig. 1). Direct binding of ATG4B to GABARAP and GABARAPL1, depending on a C-terminal LIR motif, is important for maintaining these diffusely localized pools [25]. Further studies are needed to elucidate the interplay between the centrosomal and diffusely located pools of GABARAP and GABARAPL1 and how this impacts on regulation of autophagosome formation.

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LC3 for transport and selective autophagy

As the full name suggests, microtubule-associated protein light chain 3 (MAP1LC3) was first identified as a new light chain for MAP1 [54]. Consistent with a role in transport of autophagosomes along microtubules, LC3A and -B interact via a LIR motif with the Rab7 effector FYVE and coiled coil domain-containing 1 (FYCO1) [55] (Fig. 1). FYCO1 is involved in facilitating kinesin-dependent plus-end movement of autophagosomes and late endosomes [56]. Neurons require long distance transport of autophagosomes along axons to the cell body where most of the lysosomes reside. The JNK-interacting, motor scaffold protein JIP1 promotes both plus-end and minus-end transport. Binding of LC3B to a LIR motif in JIP1 promotes a phosphorylation-dependent switch within JIP1 to promote transport of autophagosomes to the perinuclear region [57] (Fig. 1). An intact LIR motif in JIP1 is required for efficient autophagosome–lysosome fusion in neurons [57].

To summarize, LIR-containing proteins involved in autophagosome formation, that is, the core autophagy proteins, interact with a GABARAP subfamily member (presumably GABARAP) on the surface or rim of the growing phagophore. LIR proteins involved in the fusion step similarly have preference for the GABARAP subfamily. Most of the LIR-containing proteins with preference for LC3 are either proteins involved in transport of autophagosomes like FYCO1 or JIP1, or SARs using LC3 family members in their selective autophagy process. The majority of identified SARs can bind to LC3 subfamily members [22]. As discussed below the LC3 subfamily is essential in many selective autophagy processes. However, some SARs have a preference for members of the GABARAP subfamily, for example, NIX that interacts with GABARAPL1 to induce mitophagy [58].

The Tectonin β-propeller containing protein 2 (TECPR2) is a LIR-containing ATG8 interactor binding to SEC24D and to LC3C to regulate ER exit sites and ER export enabling efficient autophagosome biogenesis [59]. Functional ER exit sites are needed for phagophore formation. A mutation in TECPR2 gives rise to the neurodegenerative disease hereditary spastic paraplegia and patient cells show reduced SEC24D and defective ER export [59].

LIR–ATG8 interactions: viruses and bacteria do it too

LIR–ATG8 interactions are not only found in vertebrates, invertebrates, fungi and plants, but also in viral and bacterial proteins. So far only a few cases are known. The cytoplasmic tail of influensa A virus M2 protein contains a conserved LIR motif binding directly to LC3. The M2 LIR is required for LC3 redistribution to the plasma membrane in virus-infected cells and important for filamentous budding and virion stability [60]. A number of viral proteins also contain LIR motifs that need to be tested for functionality [61]. *Legionella pneumophila*, a common cause of community and hospital-acquired pneumonia, inhibits autophagy by injecting the RavZ cysteine protease into the host cell. RavZ binds to lipidated ATG8s via a LIR motif, extracts LC3–PE from the membrane and irreversibly deconjugates lipidated ATG8 family proteins [62]. Likely, more will be learned in the future about LIR–ATG8 interactions employed by viruses and intracellular bacteria to combat and subvert the autophagy pathway.

LIR–ATG8 interactions occur also outside autophagy

It should be mentioned that ATG8 family also engage in interactions with LIR-containing proteins in non-autophagic processes too [63,64]. For example, the adapter proteins KBTBD6 and -7 of the Cullin-3 E3 ligase contain LIR motifs binding to GABARAP for membrane targeting of the E3 ligase complex enabling regulation of RAC1 signaling via local regulation of the abundance of the guanine exchange factor TIAM1 [65]. Another example is provided by the giant ankyrin-G which promotes GABAergic synapse stability through opposing endocytosis of $GABA_A$ receptors. This requires a super-strong LIR–GABARAP interaction of ankyrin-G with K_D in the lower nanomolar range [66].

A large majority of ATG8 interactions with other proteins involved in autophagy are mediated by one or more LIR motifs in the interacting protein [22]. Hence, the LIR motifs and interactions, particularly with cargo receptors, are discussed next.

The LIR–LDS Interaction

The LIR motif was originally mapped to a 22-residue sequence of p62 containing an evolutionarily conserved motif where both electrostatic interactions mediated by three consecutive Asp residues (DDD) followed by the hydrophobic Trp (W) residue were important for binding to LC3B [18]. The structures of p62 and yeast Atg19 peptides bound to LC3B and yeast Atg8, respectively, then revealed a W-x-x-L motif (x is any amino acid) where the aromatic residue and the hydrophobic Leu (L) residue docked into two hydrophobic pockets (HP1 and HP2) in the LIR docking site (LDS) of LC3B and Atg8 [67,68]. Further structure studies using isolated LIR peptides bound to ATG8 proteins or LIR peptides expressed as N-terminal extensions of ATG8 proteins showed that LIRs usually bind as an extended β-sheet to the LDS [22,69,70]. The LDS is a crevice formed by the

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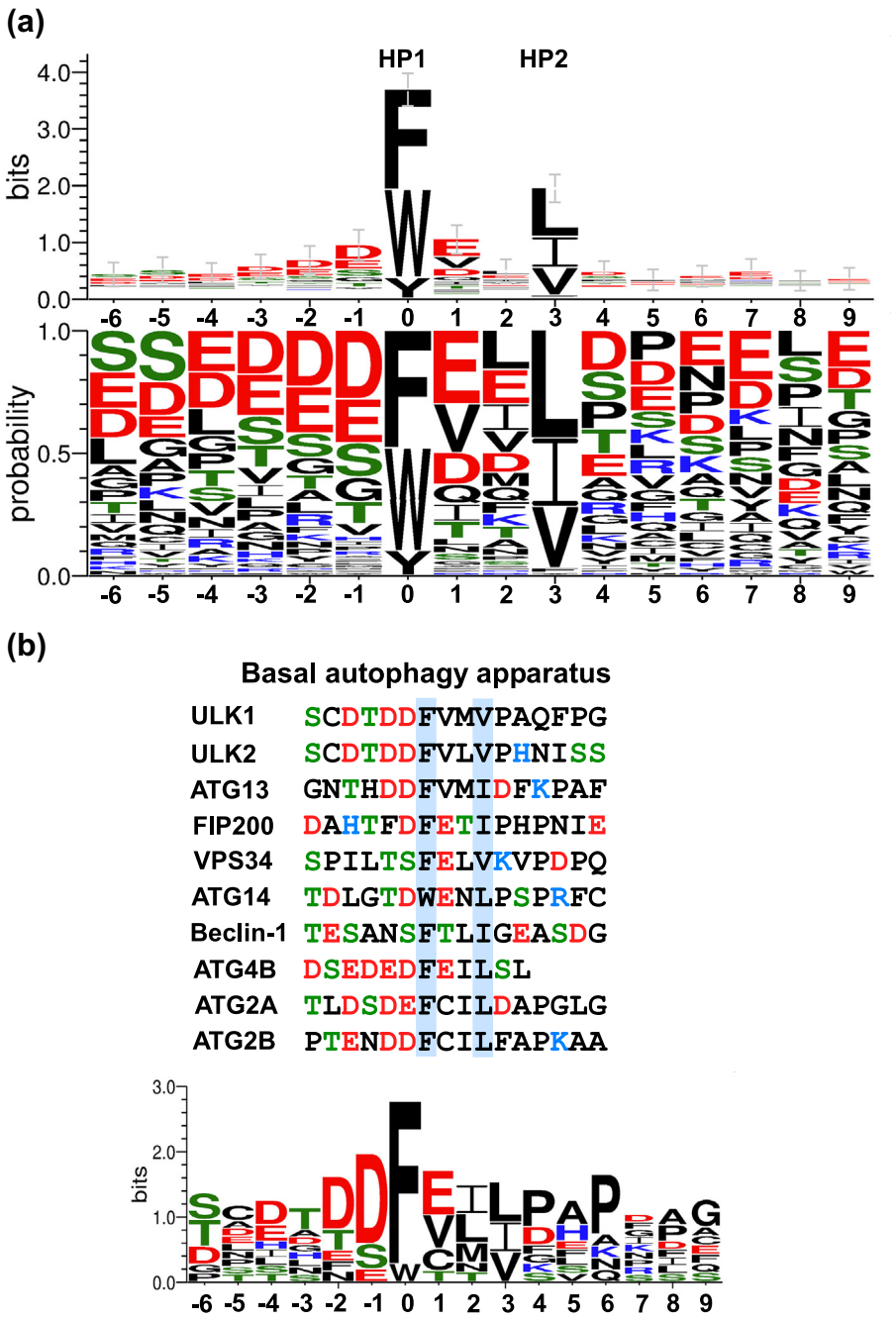

Fig. 3. LIR sequences. (A) Sequence logos based on 100 different LIR motifs with 48 LIRs with F, 42 with W and 10 with Y in position X_0 shown as information content in bits (upper panel) and residue probabilities at each position (lower panel). HP1 and HP2 indicate the residues docking into the two respective hydrophobic pockets. (B) LIR motif sequences of components of the basal or core autophagy machinery. The sequence logos were made using WebLogo 3.6.0 [195].

N-terminal arm and the UBL domain of the ATG8s harboring the two hydrophobic pockets (Fig. 2C–E).

In addition to the vital structure data from complexes of LIR peptides with ATG8 proteins or LIR peptide–ATG8 chimeras, our present knowledge of the important sequence features of the LIR motifs come from mutation analyses, combined with binding assays including two-dimensional peptide arrays (i.e., Ref. [71]). Hence, the so-called canonical LIR sequences consist of the core motif [W/F/Y]$_0$-X$_1$-X$_2$-[L/V/I]$_3$ where positions X$_0$ and X$_3$ are absolutely conserved (Fig. 3A). The core motif is flanked by N- and C-terminal sequences that also contribute both to binding affinity and specificity (see

below). The residues at positions X_{-1} to X_{-3} immediately N-terminal to the core LIR are very often acidic Asp (D) or Glu (E), or Ser (S) or Thr (T) residues that can become phosphorylated (Fig. 3A). Inspection of sequence logos based on 100 canonical LIR motifs shows that only a minority (10) contain Tyr (Y) at the absolutely conserved aromatic $[W/F/Y]_0$ position, 42 contain Trp (W) and 48 have Phe (F) at this position. W in the HP1 binds more strongly than F and Y. Changing Y for W in the NBR1 LIR increased binding affinity 7.5-fold [72]. The X_1 and X_2 positions within the core LIR are most often populated by acidic or hydrophobic residues, with the X_2 position being the most promiscuous sometimes allowing basic residues as well. A relatively high frequency of acidic residues both N-terminal, within and C-terminal to core LIR, is reflected by the fact that the LDS has a generally basic surface surrounding the deep hydrophobic pockets HP1 and -2 (Fig. 2D-E). Although a few important exceptions exist, basic residues (R and K), as well as P and G residues, are usually not found in the X_1 and X_2 positions of the core LIR. The selection against G and P, which will interfere with the β-sheet structure of the core LIR and against the basic K and R residues, is also corroborated by two dimensional peptide array mutation analyses [23,24,53,55,73].

As noted above, in the first study describing the LIR [18], the importance of acidic residues immediately preceding the core LIR was shown. Since then, structural studies have documented that these N-terminal acidic residues engage in electrostatic interactions with basic residues in the LDS of GABARAP and LC3 proteins [22,53,69,70]. However, due to the understandable focus on the core LIR signature, there is unfortunately a tendency to ignore the importance of the sequences immediately flanking the core LIR motifs, particularly C terminal to the core LIR. This may perhaps have been strengthened by the fact that one of the first LIR–LDS structures published was that of yeast Atg19–Atg8, and Atg19 has the core LIR WEEL at its extreme C terminus [68]. However, from recent structural analyses, it has become clear that a number of ATG8 interacting proteins contain C-terminal extended LIRs important for binding strength and specificity. This is the case for the human autophagy adapter ALFY (WDFY3) [74], FYCO1 [55,75,76], giant ankyrin-B (AnkB) and -G (AnkG) as well as the ERphagy receptor FAM134B [77]. Very recently, Wirth *et al.* [53] showed that also ULK1 and ATG13 LIRs have C-terminally extended LIRs important for specifying their preference for binding to GABARAP and GABARAPL1. Hence, when analyzing LIR motifs, it is important to consider that some LIR sequences may extend 5–6 residues N-terminal and 9–10 residues C-terminal to the core LIR, making the entire LIR about 20 amino acids long. This is particularly important for structure and affinity studies to avoid that too short LIR peptides are being used in the experiments. For the C-terminally extended LIRs of the AnkB, AnkG, FAM134B and FYCO1, an amphipathic α-helix immediately C-terminal to the core LIR stabilizes the binding. For FYCO1, this is a very short one-turn helix whereas for the ankyrins and FAM134, the helix has 3 helical turns and is 10 amino acids long [77]. This vastly stabilizes the LIR–LDS interaction. This is reflected in super-strong binding affinities. AnkB binds to all ATG8s with K_Ds from 0.2 to 10 nM, and FAM134B binds to GABARAP with a K_D of 0.27 nM [77]. The binding affinities measured for LIR peptides bound to ATG8 proteins using isothermal calorimetry, NMR or biolayer interferometry generally lie in the lower micromolar range for most LIR–LDS interaction [44,53,72,78].

LIR–LDS specificity determinants for binding to GABARAP or LC3 subfamily proteins

The existence of seven human ATG8 proteins prompts the question about redundancy *versus* selectivity in binding of different LIR-containing proteins to the different family members. This is of course linked to the question about redundant *versus* specific functions of the different ATG8 proteins. From the published literature, it is clear that most LIR-containing proteins bind best to GABARAP and GABARAPL1. Some bind almost exclusively to GABARAP family proteins, and a minority show preference for binding to LC3 family proteins [23,24,44,55,65,73,74]. It has been a long-standing goal to elucidate which factors determine binding preference. A recent study focused on the core LIR motif and derived a consensus sequence $[W/F]_0$-$[V/I]_1$-X_2-V_3 for GABARAP interaction motifs (GIMs) [44]. Although many LIR-containing proteins binding preferentially to GABARAP do not have the Val or Ile at position X_1 or Val at position X_3, the study clearly show that specificity determinants may reside within the core LIR motif. The LIRs of components of the core autophagy apparatus bind preferentially to GABARAPs (Fig. 3B). This includes the LIRs in ULK1/2, ATG13 and FIP200 of the ULK1 complex [23], VPS34, Beclin-1 and ATG14 in the PI3KC3 complex 1 [24], and ATG2A and -B [29]. ATG4B also binds most strongly to GABARAP [25]. Very recently, Wirth *et al.* [53] used structure solution by x-ray crystallography, mutational analyses and affinity measurements of the ULK1, ATG13 and PCM1 LIRs in complex with GABARAP to show that specificity is determined both by residues within the core LIR and the flanking C-terminal region in combination with ATG8 subfamily-specific residues in the LDS. ULK1, ATG13 and PCM1 conform to the GIM consensus. However, not all GABARAP-specific LIRs possess a GIM. As exemplified with ULK1 and ATG13, hydrogen bonds and hydrophobic

contacts of residues C-terminal to the core LIR motifs (positions X_4–X_{10}) are important for strong binding to GABARAP and involve residues, which are not conserved between LC3 and GABARAP subfamily proteins. Positions X_{-3}, X_2, and X_4 within the ULK1 LIR motif are instrumental in regulating selective binding to GABARAP over LC3 subfamily proteins [53]. The ULK1 LIR binding to GABARAP and GABARAP-L1 with K_Ds of 50 and 48 nM, respectively, is the strongest reported for any autophagy protein. The ATG13 LIR binds with 10-fold higher K_Ds. This can be explained by ULK1 residues X_7 and X_8 having hydrophobic interactions with GABARAP LDS residues missing in the ATG13 LIR–GABARAP complex. As mentioned earlier, basic residues (K and R) are unusual in LIR motifs and selected against. However, for the PCM1 LIR, the GABARAP preference is achieved by a K residue at position X_2 of the core LIR, which prevents binding to LC3 subfamily proteins but can be tolerated by GABARAP. Replacing the K by I dramatically increased binding to LC3 subfamily members and also increased binding to GABARAP [53].

In conclusion, specificity may be determined both by residues within the core LIR and the flanking N- and C-terminal region in combination with ATG8 subfamily-specific residues in the LDS.

Atypical LIR–LDS interactions

Not all LIR sequences show the canonical core LIR consensus. Some do not have the aromatic W/F/Y_0 residue, whereas others lack the hydrophobic L/V/I_3 residue. The first atypical LIR reported is the so-called C-type LIR (CLIR) in NDP52/CALCOCO2 binding strongly and specifically to LC3C [79]. The CLIR with the core sequence ILVV lacks the aromatic residue that binds in HP1 so that only HP2 of the LDS is bound. The lack of the aromatic residue is compensated by the LVV making other hydrophobic contacts with the LDS of LC3C. If I is mutated to W, HP1 is engaged and NDP52 binds to all human ATG8s losing the specificity for LC3C [79]. The most closely related SAR TAX1BP1 has M instead of I, and this enables interaction with LC3B, LC3C, GABARAPL1 and GABARAPL2 [80]. Examples of LIR–LDS interactions where only the aromatic HP1 pocket is engaged have also been reported. Bcl-2 binds to HP1 of GABARAP with an atypical LIR in Bcl-2 with the core sequence EW^{30}D lacking the hydrophobic residue at position X_3 [81]. W^{30} sits in HP1 and the binding constant for binding to GABARAP is 25 μM. Furthermore, TRIM5α has a similar core LIR, DW^{196}E interacting with both LC3 and GABARAP subfamily members [82]. This “half LIR” is projected on the α-helical coiled coil region of TRIM5α, with W^{196} protruding into the large hydrophobic pocket HP1. HP2 is not filled but has a Q residue hovering above it [83]. This structural study expands the range of LIR motifs to include α-helical binding motifs. The binding affinity is not very strong with K_Ds of about 100 and 78 μM for LC3B and GABARAPL1, respectively [83]. However, TRIM5α forms a dimer due to the coiled coil and can bind two ATG8 molecules. This will greatly increase the binding strength.

UBA5 (ubiquitin like modifier activating enzyme 5), the E1-like enzyme for the UBL UFM1 (ubiquitin fold modifier 1), has a composite LIR/UFIM (UFM1 Interaction Motif). This motif, WGIELV, is distinct from known LIR motifs and binds both to the substrate of UBA5, UFM1, and ATG8 proteins with a clear preference for GABARAPs [84]. Structural studies show that the unique LIR of UBA5 binds to the two hydrophobic pockets with the Ile (I) residue occupying HP1 and the Val (V) placed in HP2. However, in addition, the conserved Trp (W) residue N-terminal of the LIR core sequence binds into a novel hydrophobic pocket on the surface of GABARAP proteins termed HP0 [85]. Large rearrangements of key residues including the side chains of the invariant gate-keeper residue K46 and the adjacent K/R47 in GABARAP proteins allow this mode of binding expanding the LDS to three hydrophobic pockets. K/R47, corresponding to T50 in LC3A-C, is the key residue determining specific binding of GABARAP proteins to UBA5 [85].

Regulation of the LIR–LDS interactions

Only a limited number of LIR–LDS interactions have been analyzed for effects of post-translational modifications. However, it is clear that positive regulation by phosphorylation, usually at residues N-terminal to the core LIR motif occurs. This creates new electrostatic interactions to basic residues in the LDS. Phosphomimicking mutant studies show that such mutants (S or T to D or E) bind more strongly. For example, phosphomimicking E mutations of two known ULK1 phosphorylation sites N-terminal to the core LIR of VPS34 increased the binding to GABARAP by 17-fold [24]. Similarly, phosphomimicking E mutation of a predicted ULK1 phosphorylation site in the LIR of Beclin1 also increased the binding to GABARAP [24]. Positive regulation by phosphorylation is shown for the LIR motifs of several SARs involved in mitophagy, including NIX, BNIP3 and FUNDC1 [78,86,87]. The motifs in NIX, BNIP3 and FUNDC1 are all positively regulated by phosphorylation of position X_{-1} relative to the core LIR motif. FUNDC1 induces mitophagy in response to hypoxia and has a LIR motif that is both positively and negatively regulated by phosphorylation. Under hypoxia, the LIR motif is activated by ULK1 phosphorylating S17 in position X_{-1} [87]. Under normoxia, the aromatic Y18 residue in the core motif (Y^{18}EVL) is phosphorylated by Src and the

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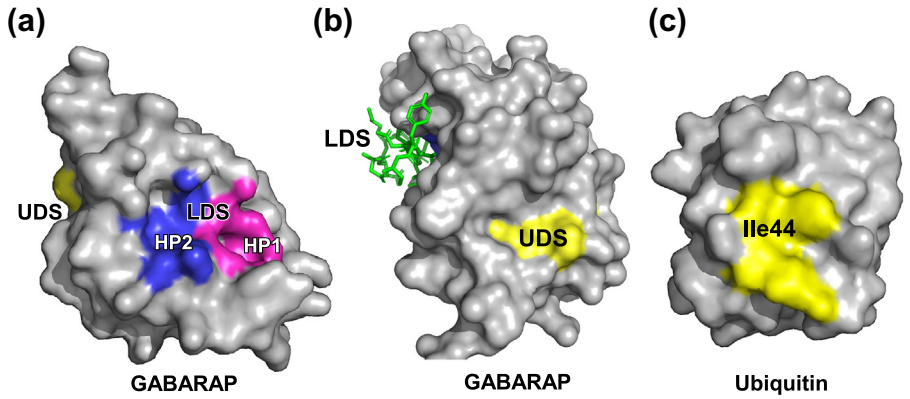

Fig. 4. The UDS and LDS lie on opposite surfaces of the ATG8 proteins. (A) GABARAP surface structure with the location of the LDS (with HP1 and HP2) indicated relative to the UDS (PDB: 3WIM). (B) GABARAP with the ALFY LIR peptide bound to the LDS displayed to highlight the location of the UDS (PDB: 3WIM). (C) For comparison, ubiquitin (PDB: 2MVS), with the Ile44 hydrophobic patch where UIM helical domains bind, is shown.

upstream residue S13 by CK2, and this has a negative effect on the binding [87]. TBK1 is an essential kinase in selective autophagy, and among its autophagy substrates are p62, OPTN, STX17 and RAB7A [11,88–90]. However, the only LIR motif shown to be regulated by TBK1 so far is the motif in OPTN, activated by a phosphorylation of S177 in position X_{-1} relative to the core LIR [90]. Much less is known about regulation of the LDS in ATG8 proteins by post-translational modifications, but the T50 residue in the region surrounding the LDS in LC3B is phosphorylated by STE20-like kinases (STK) 3 and 4 [91]. Two residues in the same region (K49 and K51) are also acetylated in the nucleus by p300 to inhibit its association with autophagy proteins. Export from the nucleus in response to starvation depends on deacetylation by SIRT1 [92].

Most *in vitro* binding studies with ATG8 proteins are performed with soluble, unlipidated ATG8s. However, in cells, most of the functionality of ATG8 proteins are connected to their membrane-bound PE-conjugated form. We know presently very little about differential binding preferences between the unlipidated, soluble and the membrane-bound lipidated forms. However, a recent study of mammalian ATG4A–D proteins shows that they are completely dependent on the C-terminal LIR motif for delipidation of ATG8 proteins [93]. A model is proposed where ATG4B effectively primes the ATG8 proteins, cleaving the soluble forms in a LIR-independent manner, whereas delipidation is an inherently slow and completely LIR-dependent process than can be performed by all ATG4 homologs [93]. More studies like this, addressing the binding to lipidated ATG8s, are required to determine if there are distinct regulatory mechanisms acting on these interactions in cells.

LIR-Independent ATG8 Interactions: The UIM–UDS Interaction

It has been known for some time that some proteins bind to ATG8 proteins in an LIR–LDS-independent manner [94–96]. The proteasomal ubiquitin receptor, RPN10, was shown by the Vierstra group to act as a cargo receptor for autophagic degradation of the proteasome in *Arabidopsis* [96]. RPN10 does not contain a LIR but interacts with ATG8s. Very recently, the same group reported that RPN10 and a group of other ATG8-interacting proteins exploit ubiquitin-interacting motif (UIM)-like sequences for high-affinity binding to an alternative ATG8 interaction site called the UIM-docking site (UDS) [97]. The UDS is on the other side of the ATG8 molecule relative to the LDS (Fig. 4). The presence of two well-separated binding surfaces in ATG8 proteins enables simultaneous binding of a LIR-containing protein to the LDS and a UIM-containing protein to the UDS (Fig. 4). This was demonstrated by simultaneous binding of *Arabidopsis* RPN10 to the UDS and DSK2 to the LDS of ATG8e [97].

The UIM domains are about 20-amino-acid-long amphipathic α helical domains first found in RPN10/S5a and subsequently in deubiquitinating enzymes USP28 and ataxin-3, proteins involved in endocytosis such as Vps27/Hrs, STAM and EPS15, as well as in the DNA repair protein RAP80. The UIM domain binds to the hydrophobic I44 patch of ubiquitin [98]. Isothermal calorimetri measurements showed the K_D values to be in the lower micromolar range for the UIM–UDS interactions for some of the plant proteins tested. This is very similar to many LIR–LDS interactions [44,53,72,78], but surprising relative to the lower-affinity binding often seen for

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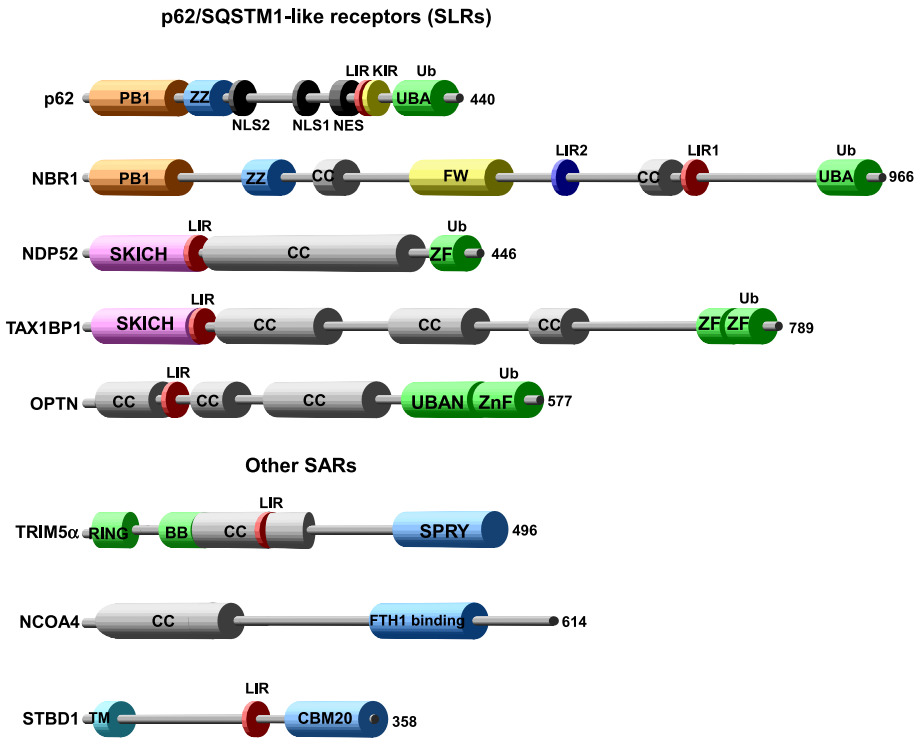

Fig. 5. Domain architecture of soluble SARs. Shown are the domain architecture of the five mammalian SLRs and three other selected SARs involved in selective autophagy of viral capsids (TRIM5α), ferritin (NCO4A), and glycogen (STBD1), respectively. Indicated motifs and domains: PB1, Phox and Bem1 domain; ZZ, ZZ-type zinc finger domain; CC, coiled-coil domain; NLS, nuclear localization signal; NES, nuclear export signal; LIR, LC3 interacting region; KIR, KEAP1 interacting region; UBA, ubiquitin-associated domain; FW, four tryptophan domain; SKICH, SKIP carboxyl homology domain; ZF, zinc finger domain; UBAN, ubiquitin binding in ABIN and NEMO domain; RING, RING finger domain; BB, B-box domain; SPRY, SPRY domain; CBM20, family 20 carbohydrate-binding module domain; TM, transmembrane domain. Boxes indicating ubiquitin binding domains are colored green.

UIM peptide interaction with ubiquitin with K_Ds in the high micromolar range (150–300 μM to even mM) [99].

Of the 204 ATG8 interactors identified in yeast and *Arabidopsis* in this study, 20% bound to the UDS [97]. It will be interesting to see whether a similar percentage of human ATG8 interactors use the UIM–UDS interface. When human LC3A and GABARAP were tested against 28 human UIM candidates, six bound to either GABARAP or LC3A or both. Epsin-1,-2, -3 and Rabenosyn bound both ATG8s, whereas Ataxin-3 and Ataxin-3L interacted only with GABARAP, showing that only a subset of UIM containing proteins may bind ATG8 proteins [97]. As the consensus sequence derived in this initial study is rather broad and derived from only 17 UIMs in 14 proteins of which several proteins are closely related, it is too early to know if it will be of predictive value as such. UIMs are quite degenerate sequences of about 20 amino acids presented as an amphipathic α helix. Structural studies of UIM peptides bound to ATG8s at the UDS are needed to reveal the crucial interacting residues.

Another intriguing point that we note about the UDS is that it overlaps with the substrate interaction site of ATG4 as determined in the structure studies by Satoo *et al*. [100]. Also, we found recently that ATG4B interacts via a C-terminal LIR motif with ATG8 proteins too [25]. Hence, when binding to ATG8s, ATG4B may simultaneously block both the LDS and the UDS. ATG4B binds very strongly to GABARAP and has a role in stabilizing a pool of GABARAP to regulate autophagy this way [25]. When ATG4B is knocked out, GABARAP is degraded by the proteasome. We speculate that the UDS may then be recognized by RPN10 to act as a receptor for proteasomal degradation of GABARAP, even without the need for E3 ligase-mediated ubiquitination of GABARAP.

The results from *Arabidopsis* and yeast strongly suggest that the UIM–UDS interaction may be widely employed to dock autophagy adaptors and receptors to ATG8 proteins. The hunt will now surely be on to identify and characterize human proteins interacting with the UDS. These may also be found among proteins not identified as having UIM domains.

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LIR-Independent ATG8 Interactions: The Cardiolipin and Lamin B1 Interactions

The inner mitochondrial membrane lipid cardiolipin becomes externalized upon damaging mitochondria using rotenone, staurosporine, 6-hydroxydopamine and other pro-mitophagy stimuli in cortical neurons and SH-SY5Y cells. The surface exposed cardiolipin acts as an "eat me" signal for the damaged mitochondria recruiting LC3B through binding to the R10 and R11 residues in the N-terminal arm of LC3B. This recruitment is essential to mediate a PINK1/Parkin-independent mitophagy [101]. The lipid ceramide also binds to LC3B to mediate a type of mitophagy resulting in autophagic cell death. The binding is dependent on lipidation and on intact I35 and F52 residues of the LDS of LC3B [102]. Another interaction requiring R10 and R11 in the N-terminal arm of LC3B is the binding to Lamin B1 during nuclear exodus and autophagic degradation of Lamin B1 occuring upon induction of oncogene-induced senescence as a tumor suppressive mechanism [103].

Soluble SARs: p62/SQSTM1-like Receptors (SLRs)

The mammalian soluble SARs consists of the p62/SQSTM1-like receptors (SLRs) [104], the ferritinophagy receptor NCOA4 [105,106], the glycophagy receptor STBD1 [107], the ribophagy receptor NUFIP1 [108], and several TRIM family E3 ligases that also can act as autophagy receptors or mediators of selective autophagy [109,110] (Fig. 5; Table 1). In yeast, Cue5, containing the ubiquitin-binding CUE domain, is known to act as a the only SAR for degradation of ubiquitinated protein aggregates, and it has been suggested that its putative mammalian homolog, Tollip, is also acting as a SAR [111]. Among the soluble SARs, the most studied are the SLRs, including p62, NBR1, NDP52, TAX1BP1 and OPTN. They were given the name by Vojo Deretic to emphasize that they can also be considered as a new class of innate immunity receptors in analogy to the pattern recognition receptors (PRRs); Toll-like receptors (TLRs), Nod-like receptors (NLRs), and RIG-I-like receptors (RLRs) [104]. The SLRs recognize cargo and recruit the core autophagy apparatus to the cargo to induce formation of mPAS structures and phagophores. They then attach themselves and their cargo firmly to the phagophore through LIR-mediated interactions with lipidated ATG8 proteins. OPTN is under normal conditions degraded by the proteasome, and activation of its LIR motif depends on phosphorylation of S177 by TBK1 [90]. The other SLRs are degraded by selective autophagy also when they are not bound to any cargo. In response to starvation, these SLRs, but not OPTN, are rapidly degraded by an autophagy pathway that does not depend on mTOR inhibition [112]. This occurs through an endosomal micro-autophagy pathway, but the exact mechanism is not known. The degradation depends on LIR motifs and lipidation of ATG8 family proteins, but the ULK1 complex and the VPS34 complex are not required/involved [112]. The cargoes for SLR-mediated selective autophagy are highly diverse. For instance, p62 is involved in degradation of misfolded proteins, protein aggregates, damaged organelles, intracellular bacteria, viral capsids, and also specific signaling proteins or protein complexes (Table 1). SLR cargoes are commonly ubiquitinated, and all SLRs have ubiquitin binding domains essential for cargo recognition (Fig. 5). However, cargo recognition by SLRs can also occur by direct interaction with a substrate, that is, p62 binding to KEAP1 [113,114], or to other "eat me" signals than ubiquitin decorating the substrate. Examples are galectins on vesicles containing intracellular bacteria [115], damaged endosomes or lysosomes [116], and NIPSNAPs on damaged mitochondria [117]. However, galectins are not simply passive tags as lysosomal damage, recognized by galectins, leads to association of galectin-8 with and inhibition of mTOR activity via the Ragulator-Rag GTPase signaling machinery [118].

The most striking feature of p62 is its ability to polymerize. The N-terminal PB1 domain of p62 can self-interact via electrostatic interactions [119,120]. The most important interaction is formed between K7 in one PB1 domain and D69 in another. Long polymeric chains can be formed by head-to-tail interactions. *In vitro* such polymerization of p62 results in the formation of flexible and helical filaments with a diameter of 15 nm [121]. When mixed with ubiquitin chains, polymerization of p62 leads to phase separation of p62 into globular structures referred to as condensates or droplets [122,123]. These structures have previously most often been referred to as p62 bodies [124]. In cells, polymerization of the PB1 domain is essential for degradation of p62 by selective autophagy, and it is needed for the formation of p62 bodies [17,18]. Mammalian p62 and NBR1 are evolutionary related [125]. They have a similar domain structure with an N-terminal PB1 domain followed by a ZZ type zinc finger and a C-terminal ubiquitin binding UBA domain (Fig. 5) [119]. They also have a LIR motif that is essential for their degradation by selective autophagy [18,126]. In p62 this motif partially overlaps with a KEAP1 interacting KIR motif [113,114]. NBR1 is twice the size of p62 and contains several domains that are absent in p62 including a coiled-coil domain responsible for NBR1 dimerization, an evolutionary conserved FW (four Trp) domain and an amphipathic helix located adjacent to the C-terminal UBA domain [125,126].

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Most non-metazoan species have a single p62/NBR1 orthologue that is more similar to NBR1 than to p62 [125]. Based on sequence similarity and conservation of domain architectures (e.g., FW domain) the *Arabidopsis thaliana* orthologue was named AtNbr1 [125]. However, it is important to keep in mind that the non-metazoan orthologues are neither p62 nor NBR1, but related to both these proteins both in sequence and function. Presumably, a gene duplication in the metazoan lineage initiated the evolution of current p62 and NBR1 in metazoans [125]. Some important functions of mammalian p62 are evolutionary conserved, and the most striking example is PB1-mediated polymerization. Nbr1 from *A. thaliana* is degraded by selective autophagy in a LIR-dependent manner. Similar to p62, AtNbr1 has an N-terminal PB1 domain that can polymerize, and this ability to polymerize is necessary for its degradation by autophagy [125]. More studies are needed to verify that the ability to polymerize is a general feature of non-metazoan p62/Nbr1 orthologues. What we know so far suggest that the presence of one p62/Nbr1 orthologue capable of polymerizing is an essential, evolutionary conserved feature in selective autophagy. Notably, insects and nematodes that have lost Nbr1 retain a polymeric p62.

The formation of p62 bodies depends on binding of the UBA domain of p62 to ubiquitin chains. This binding is regulated by phosphorylation. The unphosphorylated UBA domain of p62 has a low affinity for ubiquitin, but phosphorylation of S403 by TBK1 [89,127] or S407 by ULK1 [128] strongly increases this affinity. *In vitro*, the phase separation of p62 with ubiquitin is increased by a phosphorylation-mimicking point mutation (S403E) [122]. It is not known if ubiquitin has to be covalently attached to any particular cargo to induce p62 body formation, but ubiquitination of p62 itself on K420 is shown to induce p62 body formation [129,130]. Ubiquitin binding of p62 is inhibited by homodimerization of the UBA domain [131], and the mechanism of K420 ubiquitination is to release the UBA domain from this inhibition. Peng *et al*. [130] found that accumulation of ubiquitinated cargoes mediated binding of p62 to the ubiquitin E2 ligases UBE2D2 and UBE2D3. They catalyzed mono-ubiquitination of p62 on Lys420. The E2 enzymes bound to residues 294–320 of p62 named E2-interacting region (EIR). Another important regulation of p62 is the phosphorylation of S349 to promote binding of the KIR motif in p62 to KEAP1 and recruitment of the associated E3 ligase Cullin-3 [132]. The recruited KEAP1/Cullin-3 complex promotes p62 body formation by ubiquitination of K420 [129]. The binding of p62 to KEAP1 also activates the NRF2 signaling pathway [113,114]. Activation of NRF2 strongly induces transcription of p62 and this creates a positive feedback loop that contributes to the induction of p62 bodies [113].

An important question is whether these p62-ubiquitin condensates, or p62 bodies, directly act as platforms for assembly of phagophores, or whether they have to be sorted into smaller entities, potentially with a different structure, before they are degraded? p62 bodies formed in the nucleus are not degraded by selective autophagy, but these structures are strikingly similar to p62 bodies formed in the cytoplasm [133]. The degradation of cytoplasmic p62 bodies by selective autophagy may therefore be regulated, as seen for DALIS in dendritic cells, which may persist for several hours before their degradation is initiated [134]. It is essential to study the specific roles of recruited scaffold proteins like ALFY, huntingtin and WDR81 shown to facilitate both formation and degradation of p62 bodies by autophagy [133,135–137].

The Other SLRs

Similar to p62, NBR1 has a single LIR motif required for its own degradation by selective autophagy [126]. Degradation of NBR1 is not dependent on p62, but in response to stress, NBR1 binds to and co-localizes with p62 in p62 bodies [126]. The direct binding of NBR1 to p62 via their PB1 domains is required for this co-localization [119]. The number of NBR1 molecules in a cell is generally much lower than the number of p62 molecules [138], and there is no evidence that NBR1 prevents polymerization of p62 *in vivo*. However, NBR1 binds to the basic surface of the PB1 domain in p62, and this binding blocks further polymerization of p62. It is therefore possible that NBR1 regulates the length of p62 polymers. *In vitro*, NBR1 does not form droplets in the absence of p62, but it is recruited into p62 droplets and stimulates the formation of condensates of p62 and ubiquitin [123]. There is also evidence that NBR1 may contribute to the formation of p62 bodies [126]. NBR1 plays an important role, together with p62, in several autophagy processes including aggrephagy [126] and pexophagy [139]. A feature that is unique for metazoan NBR1 orthologues is a membrane binding amphipathic helix located just N-terminal to the UBA domain [140]. When overexpressed in cells, NBR1 uses this domain to form vesicle clusters, and the contents of these clusters, including peroxisomes, are then degraded by selective autophagy [139]. The low endogenous level of NBR1 has limited studies of endogenous NBR1, but further studies are required to establish the importance of NBR1 membrane binding in selective autophagy.

The SLRs NDP52 and TAX1BP1 are evolutionary related [80]. These paralogues have a similar domain architecture with an N-terminal SKICH domain, a central coiled-coil region and C-terminal ubiquitin binding zinc fingers (UBZs; Fig. 5). Both

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proteins have an atypical C-LIR motif located between the SKICH domain and the coiled-coil region. It is reported that NDP52 has an additional LIR in its coiled-coil region (Y204–WETE) [141], but a proposed LIR in the SKICH domain of NDP52 and TAX1BP1 is probably not accessible as it is buried in the structure [79]. Most studies on NDP52 have focused on its important roles in degradation of damaged mitochondria and intracellular bacteria. In response to mitochondria depolarization, activation of the PINK1-Parkin pathway triggers a ubiquitin-dependent recruitment of SLRs. The relative importance of SLRs may be context dependent, but several recent studies indicate an essential, yet redundant role for NDP52 and OPTN in this mitophagy pathway [142,143].

Intracellular bacteria are rapidly ubiquitinated in the cytoplasm, unless they have developed a strategy to avoid this. Then SLRs like OPTN, NDP52, p62 and TAX1BP1 are recruited and may have redundant roles [80,90,144,145]. In contrast, the role of NDP52 in the degradation of *Salmonella*-containing vacuoles (SCVs) is highly specific and does not involve ubiquitin. Instead, damaged SCVs transiently expose Galectin-3, -8, and -9 on their surface. NDP52 binds directly to Galectin-8 on damaged SCVs, and this induces their clearance by selective autophagy [115]. The atypical C-LIR motif in NDP52 binds preferentially to LC3C [79], and NDP52 uses this motif to attach Galectin-8 positive SCVs to the phagophore. Less is known about the autophagy roles of TAX1BP1, but a recent study reported an early role for LAMTOR1/LAMTOR2 in recruiting TAX1BP1 to bacteria-containing endosomes [146]. Furthermore, in xenophagy of ubiquitinated bacteria, TAX1BP1 binds to Myosin VI, and this facilitates the fusion of the autophagosome with the lysosome [80].

The SLR OPTN has important roles in mitophagy, xenophagy and aggrephagy. In all these processes, an essential step is the phosphorylation of its LIR motif (S177) and UBAN domain (S473) by TBK1 [90,142,147,148]. This phosphorylation, following the recruitment of OPTN to a selected cargo, strongly increases its affinity for ATG8 proteins and polyubiquitin. Since all SLRs interact with ubiquitin, this means that they are often recruited to the same cargo. They may have both specific and redundant roles, but it is not well understood if and how the SLRs work together in selective autophagy processes. In xenophagy, linear ubiquitin formed by LUBAC (linear ubiquitin assembly complex) is responsible for a specific recruitment of OPTN and NEMO [149,150]. NEMO then induces pro-inflammatory signaling, while OPTN participates in xenophagy. The recruitment of p62 and NDP52 does not depend on LUBAC, and these SLRs are presumably recruited by a different type ubiquitin chain. On ubiquitinated bacteria, SLRs accumulate on patches, and patches with p62 or NBR1 are separate from those containing NDP52 [151], while NDP52 and OPTN are on the same microdomains [90]. This may reflect that SLRs have a different preference for ubiquitin linkages present on the bacteria and are therefore recruited to different patches on the bacteria. However, an alternative explanation is that p62 and NBR1 forms a similar type of condensate on the bacteria as when located in “free” p62 bodies, and that this excludes a co-localization with NDP52 or OPTN. It is not known if NDP52 or OPTN have LLPT properties needed to form condensates/droplets.

Other Soluble SARs

Several of the members of the TRIM family of E3 ligases, like TRIM5α (Fig. 5), fulfill criteria of acting as SARs. They engage cargo and bind to ATG8 family proteins to mediate autophagic degradation of the cargo. They also recruit members of the core autophagy apparatus [109,110,152,153]. We therefore chose to discuss them in the section below on recruitment of the core autophagy machinery by SARs.

Iron is required in many cellular processes, but iron overload can be toxic due to ROS production. To balance the supply and demand for iron and avoid toxicity, ferritin is used as cytosolic iron storage complex. Ferritin is a cage-like protein complex made up of 24 light (FTL) and heavy (FTH1) chain subunits which surround a micelle of hydrated Fe(III) [154]. To release iron upon demand, nuclear receptor coactivator 4 (NCOA4) acts as a SAR for degradation of ferritin [105,106]. The process is known as ferritinophagy. The level of NCOA4 protein and thereby also ferritinophagy is regulated by the intracellular iron concentration. In the presence of surplus iron the E3 ubiquitin ligase HERC2 binds to and ubiquitinates NCOA4 to induce its proteasomal degradation. NCOA4 is also degraded by basal autophagy. NCOA4 interacts with ATG8s but does not contain a canonical LIR motif. Ferritin and NCOA4 have also been shown to be degraded by an alternative autophagy pathway that requires FIP200,ATG9A, VPS34, and TAX1BP1 but not the ATG8 lipidation machinery. TAX1BP1 binds directly to NCOA4 to mediate autophagic degradation of ferritin both under basal and iron-depleted conditions [155].

The selective autophagy of ribosomes, ribophagy, was first shown in yeast [156]. NUFIP1 (nuclear FMR1 interacting protein 1) was recently identified as a mammalian ribophagy receptor that binds both to ribosomes and ATG8 proteins [108]. NUFIP1 accumulates in lysosomes upon starvation and loss of NUFIP1 prevented the depletion of ribosomal proteins caused by nutrient deprivation or mTOR

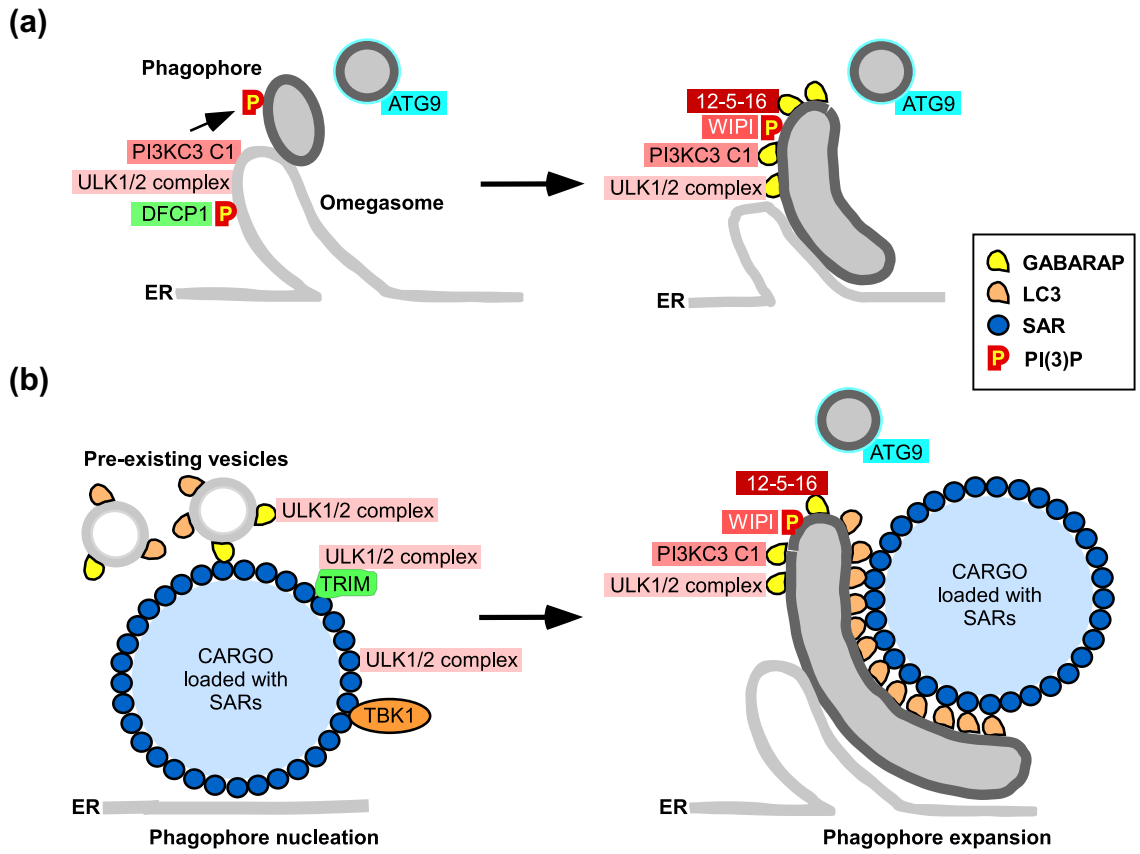

Fig. 6. Nucleation and growth of the phagophore in bulk autophagy (A) and selective autophagy (B) in mammals. In bulk autophagy (A), activation of ULK1 leads to the accumulation of the ULK1/2 and PI3KC3 complexes on DFCP1-positive ER membrane extensions (omegasomes), and this creates a platform for phagophore nucleation. An essential step is the synthesis of PI(3)P on the phagophore. This induces a recruitment of further core autophagy proteins involved in phagophore nucleation and lipidation of ATG8 proteins. In selective autophagy (B), phagophore nucleation occurs from an mPAS formed *in situ* at the SAR-coated cargo, but as in bulk autophagy, the nucleation process depends on a recruitment of the ULK1/2 and PI3KC3 complexes, and several selective autophagy processes depend on a recruitment of TBK1. How the initial complexes are recruited may differ between selective autophagy pathways, but it can be by a direct binding to the SAR or via other proteins recruited to the selected cargo (e.g., TRIM family E3 ligases). Potentially, core autophagy proteins with a LIR motif can also be recruited via pre-existing vesicles containing lipidated GABARAP. During phagophore expansion, the assembly of core autophagy proteins to the rim of the growing phagophore is stabilized by LIR interactions with lipidated GABARAP (A and B). In selective autophagy (B), the formation of multiple LIR interactions between the SAR and ATG8s lipidated to the inner surface of the phagophore is essential for the docking of the cargo to the growing phagophore.

inhibition. It is not known what is the actual ligand on ribosomes that is recognized by NUFIP1 upon mTOR inhibition. It is also important to keep in mind that in mammals ribosomes also become degraded by a kind of bystander autophagy flux during mitophagy and lysophagy [157].

Glycogen storage particles in the cytosol are degraded both by glycogen phosphorylase and by selective autophagic degradation in the lysosome called glycophagy. STBD1 (starch-binding domain 1) was identified as the glycopagy receptor. STBD1 binds to glycogen via a C-terminal glycan-binding domain and uses a LIR motif binding to GABARAPL1 to tether the glygogen cargo to the phagophore [107,158] (Fig. 5) STBD1 has a N-terminal hydrophobic region that may independently mediate targeting to the phagophore. The specific role of glycophagy in various tissues in mammals is not yet clarified, although the assumption is that glycogenolysis and glycophagy work together in mobilizing glucose from cytosolic glycogen.

The degradation of nuclear lamina by autophagy upon oncogene-induced senescence, facilitated by Lamin B1 acting as a SAR, presents a special case of a nuclear cargo being degraded (Table 1) [103]. Likely, other nuclear SARs may be identified in the future.

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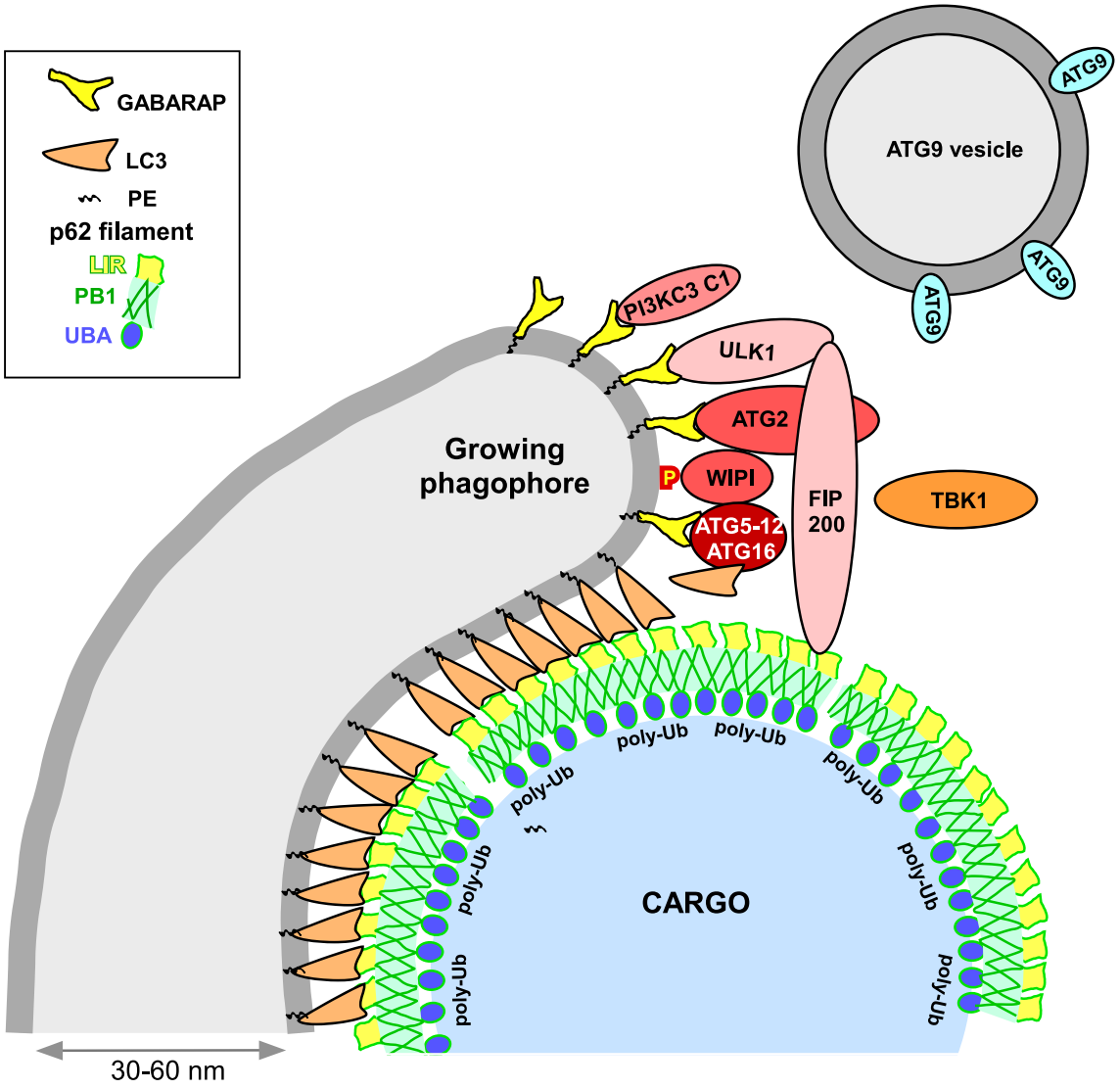

Fig. 7. Model of phagophore expansion along a p62-coated ubiquitinated cargo. At the rim of the phagophore growing out from the ER, core autophagy proteins assemble via LIR interactions with lipidated GABARAP or PI(3)P, or they are recruited by a direct binding to PI(3)P (e.g., WIPI2) or a direct interaction with p62 (e.g., FIP200). The docking of the phagophore to the cargo relies on a tight interaction between p62 and the cargo. This depends on polymerization of p62, enabling the formation of multiple interactions between the LIR motif in p62 and LC3B lipidated to the growing phagophore. This outcompetes other interactions, and other interacting core autophagy proteins like FIP200 is therefore not degraded in the process. The proteins and/or protein complexes in this cartoon are not drawn to scale.

Docking of the Cargo to the Phagophore by SARs via LIR–ATG8 Interactions

The LIR motif in SARs is essential for the targeting of the cargo to the inner (concave) side of the phagophore [19,20]. It is not known if LC3s are more used than GABARAPs as adaptors for SARs in selective autophagy, but several soluble or membrane-bound SARs can interact with LC3 family members [22,86]. The strong co-localization of p62 with LC3B in autophagosomes reflects that LC3B is commonly used as a cargo adaptor for p62, and degradation of p62 depends on LC3B [17,159,160]. Another specific example is NDP52 that in xenophagy uses a highly specific interaction with LC3C for the docking of Galectin-8-positive SCVs to the phagophore [79]. Efficient docking of any type of cargo to a phagophore is believed to rely on multiple SAR-ATG8 interactions [161,162]. Distinct from bulk autophagy (Fig. 6A), the phagophore may then grow along the selected cargo (Fig. 6B and Fig. 7). Sasha Martens coined the term “exclusive autophagy” to

describe how tight binding of SARs to the cargo on the concave side of the growing phagophore excluded other components from being sequestered. For yeast Atg19, the need for multivalency in ATG8 interactions is solved by the presence of multiple LIR motifs in the SAR. For p62 with only a single LIR motif, this is achieved by polymerization of the protein [162]. For other SLRs, it is less understood how this is resolved. NDP52, TAX1BP1 and NBR1 all have additional LIR motifs that potentially may participate in the docking, and these proteins also form dimeric or oligomeric structures via their coiled-coil motifs.

Recruitment of the Core Autophagy Machinery by SARs

Selective autophagy depends on the docking of the cargo to the phagophore, but it also relies on recruitment of upstream autophagy proteins to initiate phagophore formation [20,143,163–165]. In PINK1/Parkin-induced mitophagy induced by CCCP treatment, FIP200 and ATG9 are independently recruited before LC3, and FIP200 and ATG9 are both necessary for phagophore formation [166]. LC3 is recruited even in the absence of phagophore formation, and while not needed for phagophore formation per se, LC3 is required for the docking of the growing phagophore to the mitochondria [166]. The initial step and how individual autophagy proteins are recruited may clearly differ between selective autophagy pathways, but several recent studies suggest that SLRs are directly involved in the recruitment of upstream autophagy proteins [165,167,168] (Fig. 6B). NDP52 uses its SKICH domain to interact directly with FIP200, and this recruits the ULK complex to the cargo [165,168]. The SKICH domain in NDP52 also binds to the TBK1 adaptors SINTBAD and NAP1 [164], via a different surface of the domain [165]. The SKICH domain of TAX1BP1 similarly binds to FIP200 [165] and SINTBAD [164]. The interactions of NDP52 with FIP200-ULK1/2 and SINTBAD-TBK1 are both essential for xenophagy induced by NDP52, supporting the conclusion that the binding of NDP52 to these two kinase complexes is needed for phagophore nucleation [165]. Another recent study shows that p62 binds directly to the C-terminal Atg11 homology region of FIP200, and this recruits the ULK complex to induce phagophore formation *in situ* at the cargo [167]. A region in p62 encompassing amino acids 326–380 is sufficient for this interaction and defines the FIP200-interacting region (FIR). This region also contains the LIR and KIR motifs. The binding of p62 to LC3B or FIP200 is mutually exclusive [167]. Furthermore, the binding of p62 to FIP200 is negatively affected by a mutation of the LIR motif, while it is strongly induced by a phosphorylation-mimicking mutation of the KIR residue S349 [167]. Functionally, the FIP200 interaction is not needed for the recruitment of ULK1 to p62 bodies, but it is needed for the recruitment of ATG16L1. The FIP200 interaction also has a positive effect on degradation of p62 [167]. The use of the same region of p62 to interact with FIP200 and ATG8 proteins correlates with a model where autophagosomal degradation of upstream ATG proteins like FIP200 and the ULK complex is excluded by the stronger and polymeric binding of p62 to lipidated ATG8 proteins on the concave side of the phagophore [167] (Fig. 7). In a similar way, yeast Atg19 initially uses its C-terminal LIR motif to interact with Atg5, but this interaction is later outcompeted by the stronger Atg19-Atg8 interaction [163]. Mammalian p62, NDP52 and OPTN also interact with ATG5 [163], but it remains to be tested if these interactions overlap with the LIR–ATG8 interaction.

The scaffold protein ALFY (WDFY3) is an essential protein in p62-mediated selective autophagy that is recruited by p62 into p62 bodies and binds directly to PI3P, ATG5 and GABARAP [74,169]. By recruiting the ATG5/12/16 complex, ALFY may induce *in situ* lipidation of ATG8 proteins and thereby initiate phagophore nucleation.

As noted above, many cytoplasmic organelle cargoes like mitochondria, peroxisomes and ER have specific membrane-bound SARs that are in most cases constitutively localized to the specific cargo (Table 1). For in-depth discussion on membrane-bound SARs in mitophagy and ERphagy, see the reviews by Ganley and Wilkinson in this issue. There is so far little evidence to suggest that recruitment of upstream core autophagy proteins is a common feature among membrane-bound autophagy receptors [170], but this needs to be tested for each individual autophagy receptor. All these receptors contain a LIR motif that is essential for their function as an autophagy receptor, and the LIR motif in membrane-bound receptors is believed to have the same function as the LIR motif in SLRs. FUNDC1 is an exception among the mitophagy receptors since it also binds to ULK1, and this is essential for mitophagy induction by FUNDC1 [171]. Another example is the ERphagy receptor CCPG1 that interacts both with ATG8 proteins and FIP200 [172].

Several members of the TRIM family of E3 ligases are also recruited to autophagy substrates, and many of these interact not only with ATG8 proteins and p62, but also with the ULK1/2 and PI3KC3 complexes [82,173]. By bringing together ULK1 and Beclin-1 into a single complex, they create a platform for the initiation of phagophore formation (Fig. 6B). Several TRIMs are themselves SARs, as initially shown for TRIM5α that interacts with viral capsid proteins (cargo), ULK1, Beclin-1, p62 and ATG8 proteins [110]. TRIM5α-mediated degradation of HIV-1 p24 by selective autophagy depends on

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LIR–ATG8 interaction [110]. Other TRIM proteins recruiting ULK1 and Beclin1, and at the same time acting as SARs interacting with ATG8s, are TRIM20/MEFV and TRIM21, involved in selective autophagy of inflammasome components (NLRP3, CASP1, NLRP1) and activated IRF3, respectively [109]. TRIM16 binds to and cooperates with Galectin-3 in recognizing endomembrane damage and mediating autophagy of damaged lysosomes by recruiting core autophagy regulators ATG16L1, ULK1, and Beclin-1. The cooperation between TRIM16 and Galectin-3 also protects cells from invasion by *Mycobacterium tuberculosis* [152]. TRIM17 has a dual role in promoting selective autophagy of midbodies while inhibiting other types of selective autophagy and bulk autophagy by stabilizing an inhibitory Mcl-1–Beclin-1 complex [153]. Similar to several other of the other TRIMs regulating selective autophagy, TRIM17 interacts with ATG8s and recruits ULK1–Beclin-1 complexes [110].

Multiple interactions are formed both within and between the different core autophagy complexes. Any core autophagy protein that is recruited to the mPAS or a growing phagophore may therefore indirectly facilitate the recruitment of other core autophagy proteins. Later in the process, core autophagy proteins with a LIR motif may also be recruited to the growing phagophore via lipidated GABARAPs. SLRs and other SARs are also recruited to the expanding phagophore via lipidated ATG8s after the phagophore has been nucleated. NDP52 and OPTN were recently shown to be recruited this way creating an essential positive feedback loop. The positive feedback loop driven by ATG8–LIR interactions between SARs and phagophore amplifies the rate of autophagosome biogenesis [174]. The idea has also been proposed that before the phagophore is formed, SARs may interact with ATG8 proteins lipidated to preexisting vesicles (Fig. 6B). By recruiting GABARAP containing vesicles, upstream autophagy proteins with a LIR motif (e.g., ULK1) may then interact and participate in phagophore nucleation [20].

In mammalian cells, the location of a selected cargo that is engaged by SLRs or membrane-bound autophagy receptors in mitophagy and pexophagy, for instance, defines the organization of an mPAS (mammalian PAS). How the mPAS in selective autophagy relates to structures implicated in nonselective phagophore nucleation, including omegasomes and ER exit sites, is not well understood. This may of course depend on the cargo itself, and we need to know if omegasomes or ER exit sites are always contributing in the nucleation process. For large cargoes like *Salmonella* containing SCVs, multiple phagophores are simultaneously established, and multiple fusion events between these phagophores give rise to multiple membranes surrounding the SCV [90,145,151].

Conclusions

LIR-mediated interactions with lipidated ATG8 proteins are involved in all major steps of the autophagy pathway. Core autophagy components use these interactions for scaffolding on the phagophore, and SARs use these to dock cargo to the phagophore. SARs may also use these interactions in a positive feedforward loop for recruitment to the expanding phagophore. The mammalian LC3 and GABARAP subfamilies of ATG8s serve some specific and some redundant roles in autophagy. There is a striking preference for GABARAP proteins in membrane-scaffolding of core autophagy components. A growing body of knowledge based on structure-function studies allows us to better understand how specificity is achieved in the LIR–LDS interaction between LC3 and GABARAP subfamily proteins. These recent data also emphasize the structural flexibility of the LIR–LDS interactions. Hence, there is still a way to go before we can make good predictions about subfamily preference and strength of interactions. Other interaction surfaces than the LIR–LDS have been discovered and their relative contributions are not known. It will be interesting to see how common the UIM–UDS interaction is in mammals. This complicates the picture as do interactions using only one of the two hydrophobic pockets of the LDS.

Progress is also being made on answering how phagophore nucleation and expansion occur in selective autophagy. Several of the soluble SARs and a few of the membrane-bound SARs have been shown to interact directly with components of the core autophagy machinery presumable allowing phagophore nucleation on the cargo. Do the SARs always mediate phagophore nucleation involving omegasomes at the ER? With big cargo it is likely that several phagophores are nucleated and may expand and fuse to surround the cargo. Clearly, the elucidation of mechanisms involved in SAR-mediated phagophore nucleation and expansion will be an important field of research in the near future.

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Keywords:
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LC3;
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SAR

Abbreviations used:
ER, endoplasmic reticulum; LIR, LC3-interacting region; PE, phosphatidylethanolamine; PAS, phagophore assembly site; SAR, selective autophagy receptor; UBL, ubiquitin-like; KO, knockout; NSF, *N*-ethylmaleimide-sensitive factor; GIM, GABARAP interaction motif; UIM, ubiquitin-interacting motif; UDS, UIM-docking site; SCV, *Salmonella*-containing vacuole; SLRs, sequestosome-1-like receptors.

References

[1] N. Mizushima, M. Komatsu, Autophagy: renovation of cells and tissues, Cell. 147 (2011) 728–741.
[2] A.M. Cuervo, E. Wong, Chaperone-mediated autophagy: roles in disease and aging, Cell research. 24 (2014) 92–104.
[3] L. Galluzzi, E.H. Baehrecke, A. Ballabio, P. Boya, J.M. Bravo-San Pedro, F. Cecconi, et al., Molecular definitions of autophagy and related processes, EMBO J. 36 (2017) 1811–1836.
[4] M. Oku, Y. Sakai, Three distinct types of microautophagy based on membrane dynamics and molecular machineries, Bioessays. 40 (2018), e1800008.
[5] N. Mizushima, T. Yoshimori, Y. Ohsumi, The role of Atg proteins in autophagosome formation, Annu Rev Cell Dev Biol. 27 (2011) 107–132.
[6] Y. Ohsumi, Historical landmarks of autophagy research, Cell research. 24 (2014) 9–23.
[7] J.C. Farre, S. Subramani, Mechanistic insights into selective autophagy pathways: lessons from yeast, Nat Rev Mol Cell Biol. 17 (2016) 537–552.
[8] C.F. Bento, M. Renna, G. Ghislat, C. Puri, A. Ashkenazi, M. Vicinanza, et al., Mammalian autophagy: how does it work? Annu Rev Biochem. 85 (2016) 685–713.
[9] K. Suzuki, Y. Kubota, T. Sekito, Y. Ohsumi, Hierarchy of Atg proteins in pre-autophagosomal structure organization, Genes Cells. 12 (2007) 209–218.
[10] C.A. Lamb, T. Yoshimori, S.A. Tooze, The autophagosome: origins unknown, biogenesis complex, Nat Rev Mol Cell Biol. 14 (2013) 759–774.
[11] S. Kumar, Y. Gu, Y.P. Abudu, J.A. Bruun, A. Jain, F. Farzam, et al., Phosphorylation of syntaxin 17 by TBK1 controls autophagy initiation, Dev Cell. 49 (2019) 130–144.
[12] M.U. Hutchins, M. Veenhuis, D.J. Klionsky, Peroxisome degradation in *Saccharomyces cerevisiae* is dependent on machinery of macroautophagy and the Cvt pathway, J Cell Sci. 112 (Pt 22) (1999) 4079–4087.
[13] I.R.-E. Kim, S.; Mizushima, N.; Ohsumi, Y.; Lemasters, J. J., Autophagic degradation of mitochondria in GFP-LC3 transgenic mouse hepatocytes after nutrient deprivation, Hepatology. 40 (Suppl. 1) (2004) 291A.
[14] I. Kim, S. Rodriguez-Enriquez, J.J. Lemasters, Selective degradation of mitochondria by mitophagy, Arch Biochem Biophys. 462 (2007) 245–253.
[15] R. Leber, E. Silles, I.V. Sandoval, M.J. Mazon, Yol082p, a novel CVT protein involved in the selective targeting of aminopeptidase I to the yeast vacuole, J Biol Chem. 276 (2001) 29210–29217.
[16] S.V. Scott, J. Guan, M.U. Hutchins, J. Kim, D.J. Klionsky, Cvt19 is a receptor for the cytoplasm-to-vacuole targeting pathway, Mol Cell. 7 (2001) 1131–1141.
[17] G. Bjørkøy, T. Lamark, A. Brech, H. Outzen, M. Perander, A. Øvervatn, et al., p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death, J Cell Biol. 171 (2005) 603–614.
[18] S. Pankiv, T.H. Clausen, T. Lamark, A. Brech, J.A. Bruun, H. Outzen, et al., p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy, J Biol Chem. 282 (2007) 24131–24145.
[19] T. Johansen, T. Lamark, Selective autophagy mediated by autophagic adapter proteins, Autophagy. 7 (2011) 279–296.
[20] V. Rogov, V. Dotsch, T. Johansen, V. Kirkin, Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy, Mol Cell. 53 (2014) 167–178.
[21] M. Li, Y. Fu, Z. Yang, X.M. Yin, Measurement of the activity of the Atg4 cysteine proteases, Methods Enzymol. 587 (2017) 207–225.
[22] A.B. Birgisdottir, T. Lamark, T. Johansen, The LIR motif—crucial for selective autophagy, J Cell Sci. 126 (2013) 3237–3247.
[23] E.A. Alemu, T. Lamark, K.M. Torgersen, A.B. Birgisdottir, K. B. Larsen, A. Jain, et al., ATG8 family proteins act as scaffolds for assembly of the ULK complex: sequence requirements for LC3-Interacting Region (LIR) motifs, J Biol Chem. 287 (2012) 39275–39290.
[24] A.B. Birgisdottir, S. Mouilleron, Z. Bhujabal, M. Wirth, E. Sjottem, G. Evjen, et al., Members of the autophagy class III phosphatidylinositol 3-kinase complex I interact with GABARAP and GABARAPL1 via LIR motifs, Autophagy. (2019) 1–23.
[25] M. Skytte Rasmussen, S. Mouilleron, B. Kumar Shrestha, M. Wirth, R. Lee, K. Bowitz Larsen, et al., ATG4B contains a C-terminal LIR motif important for binding and efficient cleavage of mammalian orthologs of yeast Atg8, Autophagy. 13 (2017) 834–853.
[26] A. Kaufmann, V. Beier, H.G. Franquelim, T. Wollert, Molecular mechanism of autophagic membrane-scaffold assembly and disassembly, Cell. 156 (2014) 469–481.
[27] S. Abreu, F. Kriegenburg, R. Gomez-Sanchez, M. Mari, J. Sanchez-Wandelmer, M. Skytte Rasmussen, et al., Conserved Atg8 recognition sites mediate Atg4 association with autophagosomal membranes and Atg8 deconjugation, EMBO Rep. 18 (2017) 765–780.
[28] C. Kraft, M. Kijanska, E. Kalie, E. Siergiejuk, S.S. Lee, G. Semplicio, et al., Binding of the Atg1/ULK1 kinase to the ubiquitin-like protein Atg8 regulates autophagy, EMBO J. 31 (2012) 3691–3703.
[29] Bozic M, van den Bekerom L, Milne BA, Goodman N, Roberston L, Prescott AR, et al. A conserved ATG2-GABARAP interaction is critical for phagophore closure. bioRxiv. 2019:624627.

[30] Kriegenburg F, Ungermann C, Reggiori F. Coordination of autophagosome–lysosome fusion by Atg8 family members. Curr Biol. 2018;28:R512-R8.
[31] H. Nakatogawa, K. Suzuki, Y. Kamada, Y. Ohsumi, Dynamics and diversity in autophagy mechanisms: lessons from yeast, Nat Rev Mol Cell Biol. 10 (2009) 458–467.
[32] T. Shpilka, H. Weidberg, S. Pietrokovski, Z. Elazar, Atg8: an autophagy-related ubiquitin-like protein family, Genome biology. 12 (2011) 226.
[33] N. Jatana, D.B. Ascher, D.E.V. Pires, R.S. Gokhale, L. Thukral, Human LC3 and GABARAP subfamily members achieve functional specificity via specific structural modulations, Autophagy. (2019) 1–17.
[34] K. Tsuboyama, I. Koyama-Honda, Y. Sakamaki, M. Koike, H. Morishita, N. Mizushima, The ATG conjugation systems are important for degradation of the inner autophagosomal membrane, Science. 354 (2016) 1036–1041.
[35] T.N. Nguyen, B.S. Padman, J. Usher, V. Oorschot, G. Ramm, M. Lazarou, Atg8 family LC3/GABARAP proteins are crucial for autophagosome–lysosome fusion but not autophagosome formation during PINK1/Parkin mitophagy and starvation, J Cell Biol. 215 (2016) 857–874.
[36] L.P. Vaites, J.A. Paulo, E.L. Huttlin, J.W. Harper, Systematic analysis of human cells lacking ATG8 proteins uncovers roles for GABARAPs and the CCZ1/MON1 regulator C18orf8/RMC1 in macroautophagic and selective autophagic flux, Mol Cell Biol. 38 (2018).
[37] P. Szalai, L.K. Hagen, F. Saetre, M. Luhr, M. Sponheim, A. Overbye, et al., Autophagic bulk sequestration of cytosolic cargo is independent of LC3, but requires GABARAPs, Exp Cell Res. 333 (2015) 21–38.
[38] F. Wu, Y. Watanabe, X.Y. Guo, X. Qi, P. Wang, H.Y. Zhao, et al., Structural basis of the differential function of the two *C. elegans* Atg8 homologs, LGG-1 and LGG-2, in autophagy, Mol Cell. 60 (2015) 914–929.
[39] M. Manil-Segalen, C. Lefebvre, C. Jenzer, M. Trichet, C. Boulogne, B. Satiat-Jeunemaitre, et al., The *C. elegans* LC3 acts downstream of GABARAP to degrade autophagosomes by interacting with the HOPS subunit VPS39, Dev Cell. 28 (2014) 43–55.
[40] H. Weidberg, E. Shvets, T. Shpilka, F. Shimron, V. Shinder, Z. Elazar, LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis, EMBO J. 29 (2010) 1792–1802.
[41] D.S. Grunwald, N.M. Otto, J.M. Park, D. Song, D.H. Kim, GABARAPs and LC3s have opposite roles in regulating ULK1 for autophagy induction, Autophagy. (2019) 1–15.
[42] H. Wang, H.Q. Sun, X. Zhu, L. Zhang, J. Albanesi, B. Levine, et al., GABARAPs regulate PI4P-dependent autophagosome:lysosome fusion, Proc Natl Acad Sci U S A. 112 (2015) 7015–7020.
[43] D.G. McEwan, D. Popovic, A. Gubas, S. Terawaki, H. Suzuki, D. Stadel, et al., PLEKHM1 regulates autophagosome–lysosome fusion through HOPS complex and LC3/GABARAP proteins, Mol Cell. 57 (2015) 39–54.
[44] V.V. Rogov, A. Stolz, A.C. Ravichandran, D.O. Rios-Szwed, H. Suzuki, A. Kniss, et al., Structural and functional analysis of the GABARAP interaction motif (GIM), EMBO Rep. 18 (2017) 1382–1396.
[45] R. Marwaha, S.B. Arya, D. Jagga, H. Kaur, A. Tuli, M. Sharma, The Rab7 effector PLEKHM1 binds Arl8b to promote cargo traffic to lysosomes, J Cell Biol. 216 (2017) 1051–1070.
[46] Z. Wang, G. Miao, X. Xue, X. Guo, C. Yuan, Z. Wang, et al., The Vici syndrome protein EPG5 is a Rab7 effector that determines the fusion specificity of autophagosomes with late endosomes/lysosomes, Mol Cell. 63 (2016) 781–795.
[47] E. Itakura, C. Kishi-Itakura, N. Mizushima, The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes, Cell. 151 (2012) 1256–1269.
[48] S. Kumar, A. Jain, F. Farzam, J. Jia, Y. Gu, S.W. Choi, et al., Mechanism of Stx17 recruitment to autophagosomes via IRGM and mammalian Atg8 proteins, J Cell Biol. 217 (2018) 997–1013.
[49] J. Diao, R. Liu, Y. Rong, M. Zhao, J. Zhang, Y. Lai, et al., ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes, Nature. 520 (2015) 563–566.
[50] J.T. Kittler, P. Rostaing, G. Schiavo, J.M. Fritschy, R. Olsen, A. Triller, et al., The subcellular distribution of GABARAP and its ability to interact with NSF suggest a role for this protein in the intracellular transport of GABA(A) receptors, Mol Cell Neurosci. 18 (2001) 13–25.
[51] J. Joachim, H.B. Jefferies, M. Razi, D. Frith, A.P. Snijders, P. Chakravarty, et al., Activation of ULK kinase and autophagy by GABARAP trafficking from the centrosome is regulated by WAC and GM130, Mol Cell. 60 (2015) 899–913.
[52] J. Joachim, M. Razi, D. Judith, M. Wirth, E. Calamita, V. Encheva, et al., Centriolar satellites control GABARAP ubiquitination and GABARAP-mediated autophagy, Curr Biol. 27 (2017) 2123–2136 e7.
[53] M. Wirth, W. Zhang, M. Razi, L. Nyoni, D. Joshi, N. O'Reilly, et al., Molecular determinants regulating selective binding of autophagy adapters and receptors to ATG8 proteins, Nat Commun. 10 (2019) 2055.
[54] S.A. Kuznetsov, V.I. Gelfand, 18 kDa microtubule-associated protein: identification as a new light chain (LC-3) of microtubule-associated protein 1 (MAP-1), FEBS Lett. 212 (1987) 145–148.
[55] H.L. Olsvik, T. Lamark, K. Takagi, K.B. Larsen, G. Evjen, A. Overvatn, et al., FYCO1 contains a C-terminally extended, LC3A/B-preferring LC3-interacting region (LIR) motif required for efficient maturation of autophagosomes during basal autophagy, J Biol Chem. 290 (2015) 29361–29374.
[56] S. Pankiv, E.A. Alemu, A. Brech, J.A. Bruun, T. Lamark, A. Overvatn, et al., FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport, J Cell Biol. 188 (2010) 253–269.
[57] M.M. Fu, J.J. Nirschl, E.L. Holzbaur, LC3 binding to the scaffolding protein JIP1 regulates processive dynein-driven transport of autophagosomes, Dev Cell. 29 (2014) 577–590.
[58] I. Novak, V. Kirkin, D.G. McEwan, J. Zhang, P. Wild, A. Rozenknop, et al., Nix is a selective autophagy receptor for mitochondrial clearance, EMBO Rep. 11 (2010) 45–51.
[59] D. Stadel, V. Millarte, K.D. Tillmann, J. Huber, B.C. Tamin-Yecheskel, M. Akutsu, et al., TECPR2 cooperates with LC3C to regulate COPII-dependent ER export, Mol Cell. 60 (2015) 89–104.
[60] R. Beale, H. Wise, A. Stuart, B.J. Ravenhill, P. Digard, F. Randow, A LC3-interacting motif in the influenza A virus M2 protein is required to subvert autophagy and maintain virion stability, Cell Host Microbe. 15 (2014) 239–247.
[61] A.C. Jacomin, S. Samavedam, H. Charles, I.P. Nezis, iLIR@viral: a web resource for LIR motif-containing proteins in viruses, Autophagy. 13 (2017) 1782–1789.

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[62] A. Yang, S. Pantoom, Y.W. Wu, Elucidation of the anti-autophagy mechanism of the *Legionella* effector RavZ using semisynthetic LC3 proteins, Elife. 6 (2017).
[63] S. Subramani, V. Malhotra, Non-autophagic roles of autophagy-related proteins, EMBO Rep. 14 (2013) 143–151.
[64] L. Galluzzi, D.R. Green, Autophagy-independent functions of the autophagy machinery, Cell. 177 (2019) 1682–1699.
[65] H.M. Genau, J. Huber, F. Baschieri, M. Akutsu, V. Dotsch, H. Farhan, et al., CUL3-KBTBD6/KBTBD7 ubiquitin ligase cooperates with GABARAP proteins to spatially restrict TIAM1–RAC1 signaling, Mol Cell. 57 (2015) 995–1010.
[66] W.C. Tseng, P.M. Jenkins, M. Tanaka, R. Mooney, V. Bennett, Giant ankyrin-G stabilizes somatodendritic GABAergic synapses through opposing endocytosis of GABAA receptors, Proc Natl Acad Sci U S A. 112 (2015) 1214–1219.
[67] Y. Ichimura, T. Kumanomidou, Y.S. Sou, T. Mizushima, J. Ezaki, T. Ueno, et al., Structural basis for sorting mechanism of p62 in selective autophagy, J Biol Chem. 283 (2008) 22847–22857.
[68] N.N. Noda, H. Kumeta, H. Nakatogawa, K. Satoo, W. Adachi, J. Ishii, et al., Structural basis of target recognition by Atg8/LC3 during selective autophagy, Genes Cells. 13 (2008) 1211–1218.
[69] N.N. Noda, Y. Ohsumi, F. Inagaki, Atg8-family interacting motif crucial for selective autophagy, FEBS Lett. 584 (2010) 1379–1385.
[70] P. Wild, D.G. McEwan, I. Dikic, The LC3 interactome at a glance, J Cell Sci. 127 (2014) 3–9.
[71] T. Johansen, A.B. Birgisdottir, J. Huber, A. Kniss, V. Dotsch, V. Kirkin, et al., Methods for studying interactions between Atg8/LC3/GABARAP and LIR-containing proteins, Methods Enzymol. 587 (2017) 143–169.
[72] A. Rozenknop, V.V. Rogov, N.Y. Rogova, F. Lohr, P. Guntert, I. Dikic, et al., Characterization of the interaction of GABARAPL-1 with the LIR motif of NBR1, J Mol Biol. 410 (2011) 477–487.
[73] Z. Bhujabal, A.B. Birgisdottir, E. Sjottem, H.B. Brenne, A. Overvatn, S. Habisov, et al., FKBP8 recruits LC3A to mediate Parkin-independent mitophagy, EMBO Rep. 18 (2017) 947–961.
[74] A.H. Lystad, Y. Ichimura, K. Takagi, Y. Yang, S. Pankiv, Y. Kanegae, et al., Structural determinants in GABARAP required for the selective binding and recruitment of ALFY to LC3B-positive structures, EMBO Rep. 15 (2014) 557–565.
[75] X. Cheng, Y. Wang, Y. Gong, F. Li, Y. Guo, S. Hu, et al., Structural basis of FYCO1 and MAP1LC3A interaction reveals a novel binding mode for Atg8-family proteins, Autophagy. 12 (2016) 1330–1339.
[76] S. Sakurai, T. Tomita, T. Shimizu, U. Ohto, The crystal structure of mouse LC3B in complex with the FYCO1 LIR reveals the importance of the flanking region of the LIR motif, Acta Crystallogr F Struct Biol Commun. 73 (2017) 130–137.
[77] J. Li, R. Zhu, K. Chen, H. Zheng, H. Zhao, C. Yuan, et al., Potent and specific Atg8-targeting autophagy inhibitory peptides from giant ankyrins, Nature chemical biology. 14 (2018) 778–787.
[78] V.V. Rogov, H. Suzuki, M. Marinkovic, V. Lang, R. Kato, M. Kawasaki, et al., Phosphorylation of the mitochondrial autophagy receptor Nix enhances its interaction with LC3 proteins, Sci Rep. 7 (2017) 1131.
[79] N. von Muhlinen, M. Akutsu, B.J. Ravenhill, A. Foeglein, S. Bloor, T.J. Rutherford, et al., LC3C, bound selectively by a noncanonical LIR motif in NDP52, is required for antibacterial autophagy, Mol Cell. 48 (2012) 329–342.
[80] D.A. Tumbarello, P.T. Manna, M. Allen, M. Bycroft, S.D. Arden, J. Kendrick-Jones, et al., The autophagy receptor TAX1BP1 and the molecular motor myosin VI are required for clearance of *Salmonella* Typhimurium by autophagy, PLoS Pathog. 11 (2015), e1005174.
[81] P. Ma, M. Schwarten, L. Schneider, A. Boeske, N. Henke, D. Lisak, et al., Interaction of Bcl-2 with the autophagy-related GABAA receptor-associated protein (GABARAP): biophysical characterization and functional implications, J Biol Chem. 288 (2013) 37204–37215.
[82] M.A. Mandell, T. Kimura, A. Jain, T. Johansen, V. Deretic, TRIM proteins regulate autophagy: TRIM5 is a selective autophagy receptor mediating HIV-1 restriction, Autophagy. 10 (2014) 2387–2388.
[83] J.R. Keown, M.M. Black, A. Ferron, M. Yap, M.J. Barnett, F. G. Pearce, et al., A helical LC3-interacting region mediates the interaction between the retroviral restriction factor Trim5alpha and mammalian autophagy-related ATG8 proteins, J Biol Chem. 293 (2018) 18378–18386.
[84] S. Habisov, J. Huber, Y. Ichimura, M. Akutsu, N. Rogova, F. Loehr, et al., Structural and functional analysis of a novel interaction motif within UFM1-activating enzyme 5 (UBA5) required for binding to ubiquitin-like proteins and ufmylation, J Biol Chem. 291 (2016) 9025–9041.
[85] J. Huber, M. Obata, J. Gruber, M. Akutsu, F. Lohr, N. Rogova, et al., An atypical LIR motif within UBA5 (ubiquitin like modifier activating enzyme 5) interacts with GABARAP proteins and mediates membrane localization of UBA5, Autophagy. (2019) 1–15.
[86] A. Hamacher-Brady, N.R. Brady, Mitophagy programs: mechanisms and physiological implications of mitochondrial targeting by autophagy, Cell Mol Life Sci. 73 (2016) 775–795.
[87] M. Tagaya, K. Arasaki, Regulation of mitochondrial dynamics and autophagy by the mitochondria-associated membrane, Adv Exp Med Biol. 997 (2017) 33–47.
[88] Heo JM, Ordureau A, Swarup S, Paulo JA, Shen K, Sabatini DM, et al. RAB7A phosphorylation by TBK1 promotes mitophagy via the PINK-PARKIN pathway. Sci Adv. 2018;4: eaav0443.
[89] M. Pilli, J. Arko-Mensah, M. Ponpuak, E. Roberts, S. Master, M.A. Mandell, et al., TBK-1 promotes autophagy-mediated antimicrobial defense by controlling autophagosome maturation, Immunity. 37 (2012) 223–234.
[90] P. Wild, H. Farhan, D.G. McEwan, S. Wagner, V.V. Rogov, N.R. Brady, et al., Phosphorylation of the autophagy receptor optineurin restricts *Salmonella* growth, Science. 333 (2011) 228–233.
[91] D.S. Wilkinson, J.S. Jariwala, E. Anderson, K. Mitra, J. Meisenhelder, J.T. Chang, et al., Phosphorylation of LC3 by the Hippo kinases STK3/STK4 is essential for autophagy, Mol Cell. 57 (2015) 55–68.
[92] R. Huang, Y. Xu, W. Wan, X. Shou, J. Qian, Z. You, et al., Deacetylation of nuclear LC3 drives autophagy initiation under starvation, Mol Cell. 57 (2015) 456–466.
[93] K.J. Kauffman, S. Yu, J. Jin, B. Mugo, N. Nguyen, A. O'Brien, et al., Delipidation of mammalian Atg8-family proteins by each of the four ATG4 proteases, Autophagy. 14 (2018) 992–1010.
[94] C. Behrends, M.E. Sowa, S.P. Gygi, J.W. Harper, Network organization of the human autophagy system, Nature. 466 (2010) 68–76.

Please cite this article as: T. Johansen and T. Lamark, Selective Autophagy: ATG8 Family Proteins, LIR Motifs and Cargo Receptors..., Journal of Molecular Biology, https://doi.org/10.1016/j.jmb.2019.07.016

[95] Lin L, Yang P, Huang X, Zhang H, Lu Q, Zhang H. The scaffold protein EPG-7 links cargo/receptor complexes with the autophagic assembly machinery. J Cell Biol. 2013:in press.
[96] R.S. Marshall, F. Li, D.C. Gemperline, A.J. Book, R.D. Vierstra, Autophagic degradation of the 26S proteasome is mediated by the dual ATG8/ubiquitin receptor RPN10 in *Arabidopsis*, Mol Cell. 58 (2015) 1053–1066.
[97] R.S. Marshall, Z. Hua, S. Mali, F. McLoughlin, R.D. Vierstra, ATG8-binding UIM proteins define a new class of autophagy adaptors and receptors, Cell. 177 (2019) 766–781.
[98] K. Husnjak, I. Dikic, Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions, Annu Rev Biochem. 81 (2012) 291–322.
[99] J.H. Hurley, S. Lee, G. Prag, Ubiquitin-binding domains, Biochem J. 399 (2006) 361–372.
[100] K. Satoo, N.N. Noda, H. Kumeta, Y. Fujioka, N. Mizushima, Y. Ohsumi, et al., The structure of Atg4B–LC3 complex reveals the mechanism of LC3 processing and delipidation during autophagy, EMBO J. 28 (2009) 1341–1350.
[101] C.T. Chu, J. Ji, R.K. Dagda, J.F. Jiang, Y.Y. Tyurina, A.A. Kapralov, et al., Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells, Nat Cell Biol. 15 (2013) 1197–1205.
[102] R.D. Sentelle, C.E. Senkal, W. Jiang, S. Ponnusamy, S. Gencer, S.P. Selvam, et al., Ceramide targets autophagosomes to mitochondria and induces lethal mitophagy, Nature chemical biology. 8 (2012) 831–838.
[103] Z. Dou, C. Xu, G. Donahue, T. Shimi, J.A. Pan, J. Zhu, et al., Autophagy mediates degradation of nuclear lamina, Nature. 527 (2015) 105–109.
[104] V. Deretic, Autophagy as an innate immunity paradigm: expanding the scope and repertoire of pattern recognition receptors, Current opinion in immunology. 24 (2012) 21–31.
[105] W.E. Dowdle, B. Nyfeler, J. Nagel, R.A. Elling, S. Liu, E. Triantafellow, et al., Selective VPS34 inhibitor blocks autophagy and uncovers a role for NCOA4 in ferritin degradation and iron homeostasis in vivo, Nat Cell Biol. 16 (2014) 1069–1079.
[106] J.D. Mancias, X. Wang, S.P. Gygi, J.W. Harper, A.C. Kimmelman, Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy, Nature. 509 (2014) 105–109.
[107] S. Jiang, C.D. Wells, P.J. Roach, Starch-binding domain-containing protein 1 (Stbd1) and glycogen metabolism: identification of the Atg8 family interacting motif (AIM) in Stbd1 required for interaction with GABARAPL1, Biochem Biophys Res Commun. 413 (2011) 420–425.
[108] G.A. Wyant, M. Abu-Remaileh, E.M. Frenkel, N.N. Laqtom, V. Dharamdasani, C.A. Lewis, et al., NUFIP1 is a ribosome receptor for starvation-induced ribophagy, Science. 360 (2018) 751–758.
[109] T. Kimura, A. Jain, S.W. Choi, M.A. Mandell, K. Schroder, T. Johansen, et al., TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity, J Cell Biol. 210 (2015) 973–989.
[110] M.A. Mandell, A. Jain, J. Arko-Mensah, S. Chauhan, T. Kimura, C. Dinkins, et al., TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition, Dev Cell. 30 (2014) 394–409.
[111] K. Lu, I. Psakhye, S. Jentsch, Autophagic clearance of polyQ proteins mediated by ubiquitin-Atg8 adaptors of the conserved CUET protein family, Cell. 158 (2014) 549–563.
[112] J. Mejlvang, H. Olsvik, S. Svenning, J.A. Bruun, Y.P. Abudu, K.B. Larsen, et al., Starvation induces rapid degradation of selective autophagy receptors by endosomal microautophagy, J Cell Biol. 217 (2018) 3640–3655.
[113] A. Jain, T. Lamark, E. Sjøttem, K. Bowitz Larsen, J.A. Awuh, A. Øvervatn, et al., p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription, J Biol Chem. 285 (2010) 22576–22591.
[114] M. Komatsu, H. Kurokawa, S. Waguri, K. Taguchi, A. Kobayashi, Y. Ichimura, et al., The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1, Nat Cell Biol. 12 (2010) 213–223.
[115] T.L. Thurston, M.P. Wandel, N. von Muhlinen, A. Foeglein, F. Randow, Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion, Nature. 482 (2012) 414–418.
[116] I. Maejima, A. Takahashi, H. Omori, T. Kimura, Y. Takabatake, T. Saitoh, et al., Autophagy sequesters damaged lysosomes to control lysosomal biogenesis and kidney injury, EMBO J. 32 (2013) 2336–2347.
[117] Y. Princely Abudu, S. Pankiv, B.J. Mathai, A. Hakon Lystad, C. Bindesboll, H.B. Brenne, et al., NIPSNAP1 and NIPSNAP2 act as "eat me" signals for mitophagy, Dev Cell. 49 (2019) 509–525 e12.
[118] J. Jia, Y.P. Abudu, A. Claude-Taupin, Y. Gu, S. Kumar, S. W. Choi, et al., Galectins control mTOR in response to endomembrane damage, Mol Cell. 70 (2018) 120–135 e8.
[119] T. Lamark, M. Perander, H. Outzen, K. Kristiansen, A. Øvervatn, E. Michaelsen, et al., Interaction codes within the family of mammalian Phox and Bem1p domain-containing proteins, J Biol Chem. 278 (2003) 34568–34581.
[120] M.I. Wilson, D.J. Gill, O. Perisic, M.T. Quinn, R.L. Williams, PB1 domain-mediated heterodimerization in NADPH oxidase and signaling complexes of atypical protein kinase C with Par6 and p62, Mol Cell. 12 (2003) 39–50.
[121] R. Ciuffa, T. Lamark, A.K. Tarafder, A. Guesdon, S. Rybina, W.J. Hagen, et al., The selective autophagy receptor p62 forms a flexible filamentous helical scaffold, Cell Rep. 11 (2015) 748–758.
[122] D. Sun, R. Wu, J. Zheng, P. Li, L. Yu, Polyubiquitin chain-induced p62 phase separation drives autophagic cargo segregation, Cell research. 28 (2018) 405–415.
[123] G. Zaffagnini, A. Savova, A. Danieli, J. Romanov, S. Tremel, M. Ebner, et al., p62 filaments capture and present ubiquitinated cargos for autophagy, EMBO J. 37 (2018).
[124] T. Lamark, S. Svenning, T. Johansen, Regulation of selective autophagy: the p62/SQSTM1 paradigm, Essays Biochem. 61 (2017) 609–624.
[125] S. Svenning, T. Lamark, K. Krause, T. Johansen, Plant NBR1 is a selective autophagy substrate and a functional hybrid of the mammalian autophagic adapters NBR1 and p62/SQSTM1, Autophagy. 7 (2011) 993–1010.
[126] V. Kirkin, T. Lamark, Y.S. Sou, G. Bjorkoy, J.L. Nunn, J.A. Bruun, et al., A role for NBR1 in autophagosomal degradation of ubiquitinated substrates, Mol Cell. 33 (2009) 505–516.
[127] G. Matsumoto, T. Shimogori, N. Hattori, N. Nukina, TBK1 controls autophagosomal engulfment of polyubiquitinated mitochondria through p62/SQSTM1 phosphorylation, Hum Mol Genet. 24 (2015) 4429–4442.
[128] J. Lim, M.L. Lachenmayer, S. Wu, W. Liu, M. Kundu, R. Wang, et al., Proteotoxic stress induces phosphorylation of p62/SQSTM1 by ULK1 to regulate selective autophagic clearance of protein aggregates, PLoS Genet. 11 (2015), e1004987.

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[129] Y. Lee, T.F. Chou, S.K. Pittman, A.L. Keith, B. Razani, C.C. Weihl, Keap1/Cullin3 modulates p62/SQSTM1 activity via UBA domain ubiquitination, Cell Rep. 19 (2017) 188–202.
[130] H. Peng, J. Yang, G. Li, Q. You, W. Han, T. Li, et al., Ubiquitylation of p62/sequestosome1 activates its autophagy receptor function and controls selective autophagy upon ubiquitin stress, Cell research. 27 (2017) 657–674.
[131] J. Long, T.P. Garner, M.J. Pandya, C.J. Craven, P. Chen, B. Shaw, et al., Dimerisation of the UBA domain of p62 inhibits ubiquitin binding and regulates NF-kappaB signalling, J Mol Biol. 396 (2010) 178–194.
[132] Y. Ichimura, S. Waguri, Y.S. Sou, S. Kageyama, J. Hasegawa, R. Ishimura, et al., Phosphorylation of p62 activates the Keap1–Nrf2 pathway during selective autophagy, Mol Cell. 51 (2013) 618–631.
[133] T.H. Clausen, T. Lamark, P. Isakson, K. Finley, K.B. Larsen, A. Brech, et al., p62/SQSTM1 and ALFY interact to facilitate the formation of p62 bodies/ALIS and their degradation by autophagy, Autophagy. 6 (2010) 330–344.
[134] H. Lelouard, E. Gatti, F. Cappello, O. Gresser, V. Camosseto, P. Pierre, Transient aggregation of ubiquitinated proteins during dendritic cell maturation, Nature. 417 (2002) 177–182.
[135] M. Filimonenko, P. Isakson, K.D. Finley, M. Anderson, H. Jeong, T.J. Melia, et al., The selective macroautophagic degradation of aggregated proteins requires the PI3P-binding protein Alfy, Mol Cell. 38 (2010) 265–279.
[136] X. Liu, Y. Li, X. Wang, R. Xing, K. Liu, Q. Gan, et al., The BEACH-containing protein WDR81 coordinates p62 and LC3C to promote aggrephagy, J Cell Biol. 216 (2017) 1301–1320.
[137] Y.N. Rui, Z. Xu, B. Patel, Z. Chen, D. Chen, A. Tito, et al., Huntingtin functions as a scaffold for selective macroautophagy, Nat Cell Biol. 17 (2015) 262–275.
[138] Wang M, Herrmann CJ, Simonovic M, Szklarczyk D, von Mering C. Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. Proteomics. 2015;15:3163-8.
[139] E. Deosaran, K.B. Larsen, R. Hua, G. Sargent, Y. Wang, S. Kim, et al., NBR1 acts as an autophagy receptor for peroxisomes, J Cell Sci. 126 (2013) 939–952.
[140] F.K. Mardakheh, G. Auciello, T.R. Dafforn, J.Z. Rappoport, J.K. Heath, Nbr1 is a novel inhibitor of ligand-mediated receptor tyrosine kinase degradation, Mol Cell Biol. 30 (2010) 5672–5685.
[141] P. Verlhac, I.P. Gregoire, O. Azocar, D.S. Petkova, J. Baguet, C. Viret, et al., Autophagy receptor NDP52 regulates pathogen-containing autophagosome maturation, Cell Host Microbe. 17 (2015) 515–525.
[142] J.M. Heo, A. Ordureau, J.A. Paulo, J. Rinehart, J.W. Harper, The PINK1–PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy, Mol Cell. 60 (2015) 7–20.
[143] M. Lazarou, D.A. Sliter, L.A. Kane, S.A. Sarraf, C. Wang, J. L. Burman, et al., The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy, Nature. 524 (2015) 309–314.
[144] T.L. Thurston, G. Ryzhakov, S. Bloor, N. von Muhlinen, F. Randow, The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria, Nat Immunol. 10 (2009) 1215–1221.
[145] Y.T. Zheng, S. Shahnazari, A. Brech, T. Lamark, T. Johansen, J.H. Brumell, The adaptor protein p62/SQSTM1 targets invading bacteria to the autophagy pathway, J Immunol. 183 (2009) 5909–5916.
[146] C.Y. Lin, T. Nozawa, A. Minowa-Nozawa, H. Toh, C. Aikawa, I. Nakagawa, LAMTOR2/LAMTOR1 complex is required for TAX1BP1-mediated xenophagy, Cell Microbiol. 21 (2019), e12981.
[147] J. Korac, V. Schaeffer, I. Kovacevic, A.M. Clement, B. Jungblut, C. Behl, et al., Ubiquitin-independent function of optineurin in autophagic clearance of protein aggregates, J Cell Sci. 126 (2013) 580–592.
[148] B. Richter, D.A. Sliter, L. Herhaus, A. Stolz, C. Wang, P. Beli, et al., Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria, Proc Natl Acad Sci U S A. 113 (2016) 4039–4044.
[149] J. Noad, A. von der Malsburg, C. Pathe, M.A. Michel, D. Komander, F. Randow, LUBAC-synthesized linear ubiquitin chains restrict cytosol-invading bacteria by activating autophagy and NF-kappaB, Nat Microbiol. 2 (2017) 17063.
[150] S.J.L. van Wijk, F. Fricke, L. Herhaus, J. Gupta, K. Hotte, F. Pampaloni, et al., Linear ubiquitination of cytosolic *Salmonella* Typhimurium activates NF-kappaB and restricts bacterial proliferation, Nat Microbiol. 2 (2017) 17066.
[151] S. Mostowy, V. Sancho-Shimizu, M.A. Hamon, R. Simeone, R. Brosch, T. Johansen, et al., p62 and NDP52 proteins target intracytosolic Shigella and Listeria to different autophagy pathways, J Biol Chem. 286 (2011) 26987–26995.
[152] S. Chauhan, S. Kumar, A. Jain, M. Ponpuak, M.H. Mudd, T. Kimura, et al., TRIMs and galectins globally cooperate and TRIM16 and Galectin-3 co-direct autophagy in endomembrane damage homeostasis, Dev Cell. 39 (2016) 13–27.
[153] M.A. Mandell, A. Jain, S. Kumar, M.J. Castleman, T. Anwar, E.L. Eskelinen, et al., TRIM17 contributes to autophagy of midbodies while actively sparing other targets from degradation, J Cell Sci. 129 (2016) 3562–3573.
[154] Santana-Codina N, Mancias JD. The role of NCOA4-mediated ferritinophagy in health and disease. Pharmaceuticals (Basel). 2018;11.
[155] J.M. Goodwin, W.E. Dowdle, R. DeJesus, Z. Wang, P. Bergman, M. Kobylarz, et al., Autophagy-independent lysosomal targeting regulated by ULK1/2-FIP200 and ATG9, Cell Rep. 20 (2017) 2341–2356.
[156] C. Kraft, A. Deplazes, M. Sohrmann, M. Peter, Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease, Nat Cell Biol. 10 (2008) 602–610.
[157] H. An, J.W. Harper, Systematic analysis of ribophagy in human cells reveals bystander flux during selective autophagy, Nat Cell Biol. 20 (2018) 135–143.
[158] S. Jiang, B. Heller, V.S. Tagliabracci, L. Zhai, J.M. Irimia, A. A. DePaoli-Roach, et al., Starch binding domain-containing protein 1/genethonin 1 is a novel participant in glycogen metabolism, J Biol Chem. 285 (2010) 34960–34971.
[159] Y. Maruyama, Y.S. Sou, S. Kageyama, T. Takahashi, T. Ueno, K. Tanaka, et al., LC3B is indispensable for selective autophagy of p62 but not basal autophagy, Biochem Biophys Res Commun. 446 (2014) 309–315.
[160] E. Shvets, A. Abada, H. Weidberg, Z. Elazar, Dissecting the involvement of LC3B and GATE-16 in p62 recruitment into autophagosomes, Autophagy. 7 (2011) 683–688.
[161] J. Sawa-Makarska, C. Abert, J. Romanov, B. Zens, I. Ibiricu, S. Martens, Cargo binding to Atg19 unmasks further Atg8-

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binding sites to mediate membrane-cargo apposition during selective autophagy, Nat Cell Biol. 16 (2014).
[162] B. Wurzer, G. Zaffagnini, D. Fracchiolla, E. Turco, C. Abert, J. Romanov, et al., Oligomerization of p62 allows for selection of ubiquitinated cargo and isolation membrane during selective autophagy, Elife. 4 (2015), e08941.
[163] D. Fracchiolla, J. Sawa-Makarska, B. Zens, A. Ruiter, G. Zaffagnini, A. Brezovich, et al., Mechanism of cargo-directed Atg8 conjugation during selective autophagy, Elife. 5 (2016).
[164] T.L. Thurston, K.B. Boyle, M. Allen, B.J. Ravenhill, M. Karpiyevich, S. Bloor, et al., Recruitment of TBK1 to cytosol-invading Salmonella induces WIPI2-dependent antibacterial autophagy, EMBO J. 35 (2016) 1779–1792.
[165] B.J. Ravenhill, K.B. Boyle, N. von Muhlinen, C.J. Ellison, G. R. Masson, E.G. Otten, et al., The cargo receptor NDP52 initiates selective autophagy by recruiting the ULK complex to cytosol-invading bacteria, Mol Cell. 74 (2019) 320–329.
[166] E. Itakura, C. Kishi-Itakura, I. Koyama-Honda, N. Mizushima, Structures containing Atg9A and the ULK1 complex independently target depolarized mitochondria at initial stages of Parkin-mediated mitophagy, J Cell Sci. 125 (2012) 1488–1499.
[167] E. Turco, M. Witt, C. Abert, T. Bock-Bierbaum, M.Y. Su, R. Trapannone, et al., FIP200 claw domain binding to p62 promotes autophagosome formation at ubiquitin condensates, Mol Cell. 74 (2019) 330–346.
[168] J.N.S. Vargas, C. Wang, E. Bunker, L. Hao, D. Maric, G. Schiavo, et al., Spatiotemporal control of ULK1 activation by NDP52 and TBK1 during selective autophagy, Mol Cell. 74 (2019) 347–362.
[169] P. Isakson, P. Holland, A. Simonsen, The role of ALFY in selective autophagy, Cell Death Differ. 20 (2013) 12–20.
[170] C.E. Rodger, T.G. McWilliams, I.G. Ganley, Mammalian mitophagy—from in vitro molecules to in vivo models, FEBS J. 285 (2018) 1185–1202.
[171] W. Wu, W. Tian, Z. Hu, G. Chen, L. Huang, W. Li, et al., ULK1 translocates to mitochondria and phosphorylates FUNDC1 to regulate mitophagy, EMBO Rep. 15 (2014) 566–575.
[172] M.D. Smith, M.E. Harley, A.J. Kemp, J. Wills, M. Lee, M. Arends, et al., CCPG1 is a non-canonical autophagy cargo receptor essential for ER-phagy and pancreatic ER proteostasis, Dev Cell. 44 (2018) 217–232 e11.
[173] T. Kimura, M. Mandell, V. Deretic, Precision autophagy directed by receptor regulators—emerging examples within the TRIM family, J Cell Sci. 129 (2016) 881–891.
[174] B.S. Padman, T.N. Nguyen, L. Uoselis, M. Skulsuppaisarn, L.K. Nguyen, M. Lazarou, LC3/GABARAPs drive ubiquitin-independent recruitment of Optineurin and NDP52 to amplify mitophagy, Nat Commun. 10 (2019) 408.
[175] Korac J, Schaeffer V, Kovacevic I, Clement AM, Jungblut B, Behl C, et al. Ubiquitin-independent function of optineurin in autophagic clearance of protein aggregates. J Cell Sci. 2012.
[176] Y.C. Wong, E.L. Holzbaur, Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation, Proc Natl Acad Sci U S A. 111 (2014) E4439–E4448.
[177] F. Strappazzon, F. Nazio, M. Corrado, V. Cianfanelli, A. Romagnoli, G.M. Fimia, et al., AMBRA1 is able to induce mitophagy via LC3 binding, regardless of PARKIN and p62/SQSTM1, Cell Death Differ. 22 (2015) 419–432.
[178] C. Van Humbeeck, T. Cornelissen, H. Hofkens, W. Mandemakers, K. Gevaert, B. De Strooper, et al., Parkin interacts with Ambra1 to induce mitophagy, J Neurosci. 31 (2011) 10249–10261.
[179] R.A. Hanna, M.N. Quinsay, A.M. Orogo, K. Giang, S. Rikka, A.B. Gustafsson, Microtubule-associated protein 1 light chain 3 (LC3) interacts with Bnip3 protein to selectively remove endoplasmic reticulum and mitochondria via autophagy, J Biol Chem. 287 (2012) 19094–19104.
[180] L. Liu, D. Feng, G. Chen, M. Chen, Q. Zheng, P. Song, et al., Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells, Nat Cell Biol. 14 (2012) 177–185.
[181] T. Murakawa, O. Yamaguchi, A. Hashimoto, S. Hikoso, T. Takeda, T. Oka, et al., Bcl-2-like protein 13 is a mammalian Atg32 homologue that mediates mitophagy and mitochondrial fragmentation, Nat Commun. 6 (2015) 7527.
[182] Y. Wei, W.C. Chiang, R. Sumpter Jr., P. Mishra, B. Levine, Prohibitin 2 is an inner mitochondrial membrane mitophagy receptor, Cell. 168 (2017) 224–238 e10.
[183] Y. Zhang, Y. Yao, X. Qiu, G. Wang, Z. Hu, S. Chen, et al., Listeria hijacks host mitophagy through a novel mitophagy receptor to evade killing, Nat Immunol. 20 (2019) 433–446.
[184] J. Zhang, D.N. Tripathi, J. Jing, A. Alexander, J. Kim, R.T. Powell, et al., ATM functions at the peroxisome to induce pexophagy in response to ROS, Nat Cell Biol. 17 (2015) 1259–1269.
[185] D. Grasso, A. Ropolo, A. Lo Re, V. Boggio, M.I. Molejon, J. L. Iovanna, et al., Zymophagy, a novel selective autophagy pathway mediated by VMP1–USP9x–p62, prevents pancreatic cell death, J Biol Chem. 286 (2011) 8308–8324.
[186] F. Fumagalli, J. Noack, T.J. Bergmann, E. Cebollero, G.B. Pisoni, E. Fasana, et al., Translocon component Sec62 acts in endoplasmic reticulum turnover during stress recovery, Nat Cell Biol. 18 (2016) 1173–1184.
[187] P. Grumati, G. Morozzi, S. Holper, M. Mari, M.I. Harwardt, R. Yan, et al., Full length RTN3 regulates turnover of tubular endoplasmic reticulum via selective autophagy, Elife. 6 (2017).
[188] A. Khaminets, T. Heinrich, M. Mari, P. Grumati, A.K. Huebner, M. Akutsu, et al., Regulation of endoplasmic reticulum turnover by selective autophagy, Nature. 522 (2015) 354–358.
[189] Q. Chen, Y. Xiao, P. Chai, P. Zheng, J. Teng, J. Chen, ATL3 is a tubular ER-phagy receptor for GABARAP-mediated selective autophagy, Curr Biol. 29 (2019) 846–855 e6.
[190] H. An, A. Ordureau, J.A. Paulo, C.J. Shoemaker, V. Denic, J.W. Harper, TEX264 is an endoplasmic reticulum-resident ATG8-interacting protein critical for ER remodeling during nutrient stress, Mol Cell. 74 (2019) 891–908.
[191] H. Chino, T. Hatta, T. Natsume, N. Mizushima, Intrinsically disordered protein TEX264 mediates ER-phagy, Mol Cell. 74 (2019) 909–921.
[192] A. Orvedahl, S. MacPherson, R. Sumpter Jr., Z. Talloczy, Z. Zou, B. Levine, Autophagy protects against Sindbis virus infection of the central nervous system, Cell Host Microbe. 7 (2010) 115–127.
[193] P. Isakson, A.H. Lystad, K. Breen, G. Koster, H. Stenmark, A. Simonsen, TRAF6 mediates ubiquitination of KIF23/MKLP1 and is required for midbody ring degradation by selective autophagy, Autophagy. 9 (2013) 1955–1964.
[194] C. Pohl, S. Jentsch, Midbody ring disposal by autophagy is a post-abscission event of cytokinesis, Nat Cell Biol. 11 (2009) 65–70.
[195] G.E. Crooks, G. Hon, J.M. Chandonia, S.E. Brenner, WebLogo: a sequence logo generator, Genome Res. 14 (2004) 1188–1190.

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