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Receptor protein complexes are in control of autophagy

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In autophagic processes a variety of cargos is delivered to the degradative compartment of cells. Recent progress in autophagy research has provided support for the notion that when autophagic processes are operating in selective mode, a receptor protein complex will process the cargo. Here we present a concept of receptor protein complexes as comprising a functional tetrad of components: a ligand, a receptor, a scaffold and an Atg8 family protein. Our current understanding of each of the four components and their interaction in the context of cargo selection are considered in turn.

Keywords: autophagic cargo, ligand, receptor, scaffold protein, Atg8 family protein, phagophore

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Autophagy is a constellation of quality and quantity degradation pathways by which cells may nonselectively or selectively capture, deliver and, in most cases, digest their internal components in a homeostatic function and in response to a diverse range of cellular emergencies. The cargos include nonspecific cytoplasmic substrates, as well as vacuolar hydrolase precursors, protein aggregates, unwanted or damaged organelles and invasive microorganisms.[1-7] The signature of such a sophisticated and tightly regulated autophagic degradation pathway, is that, in selective mode, almost all (if not all) autophagic cargos destined for degradation will be processed by a receptor protein complex.[8-11] Therein lies a conundrum: How is a given autophagic cargo selected from many others, and what parameters are required to guide it to its ultimate degradation and the subsequent reuse of the degradative products by the cell? We suggest that the choice of autophagic cargo for selective macroautophagy (hereafter autophagy) is orchestrated by a functional tetrad of components: a ligand, a receptor, a scaffold and an Atg8 family protein (**Fig. 1A**).[8-11]

In autophagic terms, a ligand can be defined as a molecular entity on the surface of a cargo recognized by a receptor. In order to be ultimately degraded, cargo with ligand must go through a cascade of sequentially regulated steps involving a cohort of molecular players (e.g., receptors and Atg proteins) and intricate membrane dynamic events (e.g., autophagosome formation, and their subsequent fusion with the vacuole/lysosome membrane).[1,4,5,7,8,10-12] The specific ligands responsible for targeting various cargos for degradation by autophagy are just beginning to be revealed, but ligands for some autophagy cargos have been identified. Examples include mitochondria [addition of ubiquitin (Ub) to one or more outer membrane proteins, including VDAC1, MFN1/2 and BNIP1, by the E3 Ub ligase PARK2/PARKIN in mammals],[13-15] peroxisomes (Pex3 and Pex14 in yeasts)[16-18] or protein aggregates (Ub, mutant SOD1 or STAT5A_ΔE18 in mammals)[9,10,19-22] (**Table 1**). However, the specific ligands responsible for selective targeting of

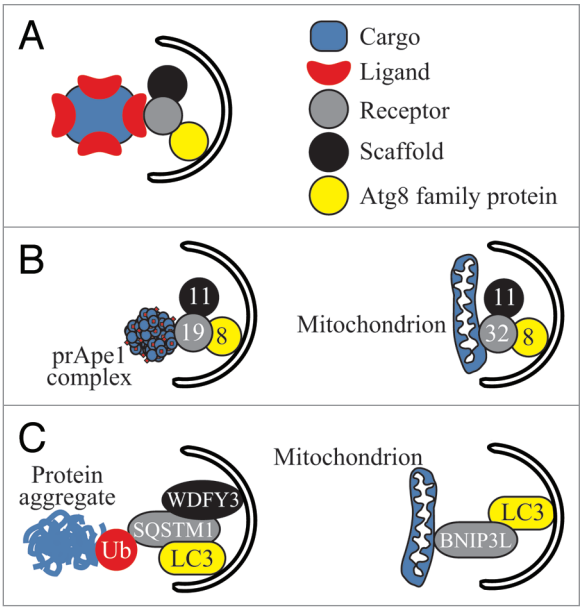

Figure 1. Receptor protein complexes in macroautophagy. (**A**) A general model of the receptor protein complex. (**B**) The Cvt pathway (left) and mitophagy (right) in yeast with their respective cargos (prApe1 complex, mitochondrion), ligand (prApe1 propeptide), receptors (Atg19 and Atg32), scaffold (Atg11) and Atg8 family protein (Atg8). (**C**) Aggrephagy (left) and mitophagy (right) in mammalian cells and their receptor protein complexes. BNIP3L might act as a "mammalian Atg32" being integral to the mitochondrial outer membrane and interacting with LC3. SQSTM1 binds to ubiquitin (Ub) conjugated to aggregated proteins and mediates their interaction with the autophagic scaffold (WDFY3) and Atg8 family protein (LC3); SQSTM1 might play a similar role during pexophagy, mitophagy and xenophagy. Note that SQSTM1 might also directly recognize several ligands for aggrephagy and xenophagy in a Ub-independent manner (**Table 1**).

certain types of autophagic cargo, such as the endoplasmic reticulum or lipid droplets, have not yet been identified.[4]

In general, components of the autophagic cargo may be either recognized directly by a receptor, or first modified with Ub by an E3 Ub ligase and then recognized by a Ub-binding receptor. Ubiquitination of cargo proteins often triggers selective autophagy in mammalian cells. However, this regulatory mechanism is not found in yeast (**Table 1**). In yeast, the best-characterized ligand is the propeptide of precursor aminopeptidase I (prApe1). This ligand is recognized by a soluble receptor, Atg19, as the first step of import of the prApe1 oligomer to the vacuole through the cytoplasm-to-vacuole targeting (Cvt) pathway (**Fig. 1B**).[23-27] Similarly, pexophagy in *Pichia pastoris* requires the Atg30 receptor that interacts with the peroxisomal membrane proteins Pex3 and Pex14.[16,17] Atg19 has some structural similarities to SQSTM1/p62 and NBR1 in mammalian cells.[9] Although SQSTM1 and NBR1 function as Ub-binding receptors for the autophagic elimination of ubiquitinated protein aggregates, organelles and bacteria, recently it was found that SQSTM1 can directly recognize some protein aggregates, bactericidal factors and viruses, like a yeast receptor, strengthening the possibility of its common origin with Atg19 (**Table 1**).[5,7,10] In contrast to the receptors of the Cvt pathway and pexophagy, the mitophagy receptors, Atg32 (yeasts), BNIP3, BNIP3L/NIX and FUNDC1 (mammals), are integral components of the mitochondrial outer membrane. However, ligand proteins recognized by Atg32, BNIP3, BNIP3L and FUNDC1 in the mitochondrial outer membrane, if they exist, have not been identified (**Fig. 1B and C**).[3,8,11-14,28-33]

It is interesting to briefly consider the evolution of the receptor mechanism with regard to bacteria. SQSTM1, CALCOCO2/NDP52 and OPTN are all required for efficient xenophagy, and they appear to bind the same bacteria at various microdomains. Specifically, CALCOCO2 and OPTN bind to the same microdomain, which is distinct from the microdomain bound by SQSTM1.[34-36] At this time we can only speculate as to the reason for involving multiple receptors, but it is possible that each one contributes a unique component or function to the autophagic process. The tissue-specificity and the distribution of receptor proteins have not been fully evaluated, but could be an important contributor to the different observations that have been reported.

The next step in selective autophagy typically involves binding of the receptor to a scaffold. In autophagic terms, a scaffold can be defined as an autophagic protein that connects a receptor with the rest of the autophagic machinery. Usually, a scaffold is a protein that organizes the autophagic machinery at the phagophore assembly site (PAS). For example, Atg11 acts as a scaffold within the Cvt pathway, pexophagy and mitophagy in yeast (**Fig. 1B**), binding Atg19/Atg34, Atg30 and Atg32, respectively.[11,12,16-18,26,27,37-39] This interaction is necessary to recruit cargo into close proximity with the autophagy machinery, and in particular an Atg8 family protein (see below). Atg11 might also act as a nonconventional tether for Atg9-containing membranes through its interaction with the GTP-bound form of the Ypt1 GTPase.[40] Bridging between the prApe1-Atg19 complex and the Atg9-containing membranes might constitute the earliest step in Cvt-specific PAS formation that is accomplished by Atg11. Atg19 contains binding sites for both Atg11 and Atg8.[12] prApe1 does not colocalize with Atg8 in the absence of either Atg19 or Atg11. Thus, Atg11 first contributes to the organization of the Cvt-specific PAS and brings prApe1-Atg19 into proximity of Atg8 at the PAS for subsequent interaction with the phagophore and selective sequestration of the prApe1-Atg19 complex. Interaction of Atg19 with Atg8 is considered to be instrumental in achieving the selectivity of this sequestration.[37,41] Similarly, Atg32 does not localize to the PAS in the absence of Atg11, whereas in wild-type cells under conditions where mitophagy is induced, Atg32 binds Atg11 and subsequently interacts with Atg8 (**Fig. 1B**).[11,12,28,29,42] In mammalian cells, WDFY3/ALFY functions as a scaffold (**Fig. 1C**), recruiting aggregated proteins

Table 1. Components of the receptor protein complexes involved in autophagy

Process	Cargo	Ligand	Receptor	Scaffold	Atg8 family protein	Refs.
Cvt pathway (yeasts)	prApe1 complex	prApe1 propeptide	Atg19	Atg11	Atg8	23, 24, 26, 27
	Ams1 complex	Ams1	Atg19, Atg34	Atg11	Atg8	38, 39
Glycophagy (mammals)	Glycogen particles	Glycogen	STBD1	-	GABARAP	58, 59
Aggrephagy (worms)	PGL granules	PGL-3	SEPA-1	EPG-2	LGG-1	60, 61
Aggrephagy (mammals)	Mutant SOD1 aggregates	Mutant SOD1	SQSTM1	-	LC3	20, 21
	STAT5A_ΔE18 aggregates	STAT5A_ΔE18	SQSTM1	-	-	22
	Ubiquitinated protein aggregates	Ub	SQSTM1, NBR1	WDFY3	LC3	43–52
Midbophagy (mammals)	Midbodies	Ub	SQSTM1	-	LC3	62
		CEP55	NBR1	-	-	63
Pexophagy (yeasts)	Peroxisomes	Pex3, Pex14	Atg30	Atg11, Atg17	-	17, 18
Pexophagy (mammals)	Peroxisomes	Pex14	-	-	LC3	64
		Ub	SQSTM1	-	LC3	65
Mitophagy (yeasts)	Mitochondria	Outer membrane	Atg32	Atg11	Atg8	28, 29
Mitophagy (mammals)	Mitochondria	Outer membrane	BNIP3, BNIP3L, FUNDC1	-	LC3, GABARAP	30–33
		Ub	SQSTM1	-	LC3	66–72
Xenophagy (mammals)	Viruses	Viral capsid proteins	SQSTM1	-	LC3	73
	Ubiquitinated bacteria	Ub	SQSTM1, CALCOCO2, OPTN	-	LC3	34–36, 74–76
	Bactericidal factors	FAU, Ub	SQSTM1	-	LC3	77
	Membrane remnants, damaged vesicles	Ub, LGALS8	SQSTM1, CALCOCO2	-	LC3	78, 79

tagged by Ub and recognized by SQSTM1 to PtdIns3P-containing membranes, and facilitating the interaction of SQSTM1 with mammalian Atg8 (LC3).[7-10,43-52] At present, there are no known scaffolds that interact with other mammalian receptors (**Table 1**).

The last player in the process, then, is an Atg8 family protein (often LC3 in mammals) recognized by the ligand-bound receptor on the phagophore membrane through a specific Atg8 family interacting motif (AIM) or LC3-interacting region (LIR). It should be noted that there are multiple Atg8 homologs in mammals, and these are grouped into two major subfamilies, LC3 and GABARAP. The in vivo binding specificities of the different family members still remains elusive but the LC3 proteins have been suggested to participate in an earlier stage of autophagy than GABARAP proteins.[53] It should also be pointed out that not all LIR-containing proteins are autophagy receptors.

The phosphorylation of autophagy receptors (e.g., Atg30, Atg32, and OPTN) might be a general mechanism for the regulation of selective autophagy.[17,35,54] The Atg8/LC3 proteins themselves are also phosphorylated, and recent studies have identified specific phosphorylation sites for protein kinase A (PKA) and protein kinase C (PKC) in the N terminus of LC3. Interestingly, this part of LC3 is involved in its binding to autophagic receptors.[55,56] It is therefore tempting to speculate that phosphorylation at the PKA and PKC sites might facilitate or prevent the interaction of LC3 with autophagic receptors such as SQSTM1. Along these lines, phosphorylation of the PKA site, which is conserved in all mammalian LC3 isoforms, but not in GABARAP, inhibits recruitment of LC3 into autophagosomes.[55]

Before concluding, we present some speculative musings that suggest the potential for biological complexity. One advantage of a receptor recruiting a scaffold is that a scaffold can specifically organize the autophagic machinery on the receptor-tagged cargos. The possibility exists that under different conditions a scaffold can be mono- or multi-specific, meaning that it could be recognized by either a single autophagy receptor (e.g., WDFY3 recognized by SQSTM1) or multiple receptors (e.g., Atg11 recognized by Atg19, Atg30, Atg32 and Atg34). Accordingly, it is feasible that the same scaffold could be recruited by multiple receptors at the same time (e.g., under starvation conditions). Moreover, the receptors may recruit multiple scaffolds (e.g., Atg30 recognizes both Atg11 and Atg17). The receptors can be soluble or membrane-bound, yet must be able to interact with their cognate scaffolds and/or Atg8 family members as well as retain the potential to form an aggregated structure under appropriate conditions (e.g., SQSTM1 involvement in the selective autophagy of bacteria, xenophagy[7,10]).

Rapid progress in autophagy research has strengthened the notion of the receptor protein complex and its role in the mechanism of selective autophagy. We anticipate the results of further studies designed to identify and characterize more autophagic cargos, ligands, receptors, scaffolds and phagophore proteins, and their respective roles under both physiological and pathological settings. Along these lines, a recent genome-wide siRNA screen aimed at identifying mammalian genes required for selective autophagy found 141 candidate genes that are required for viral autophagy, and 96 of those were also required for PARK2-mediated mitophagy.[57]

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