HHS Public Access

Author manuscript

Curr Opin Physiol. Author manuscript; available in PMC 2023 January 26.

Published in final edited form as:

Curr Opin Physiol. 2022 October ; 29: . doi:10.1016/j.cophys.2022.100590.

Mechanisms Controlling Selective Elimination of Damaged Lysosomes

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Abstract

Lysosomes are subjected to physiological and patho-physiological insults over the course of their life cycle and are accordingly repaired or recycled. Lysophagy, the selective degradation of lysosomes via autophagy, occurs upon unrepairable lysosomal membrane rupture; galectins bind to glycosylated macromolecules in the lysosome lumen, orchestrating a series of cellular responses to promote autophagic recycling of damaged lysosomes and transcriptional upregulation of lysosomal genes. Damaged lysosomes are ubiquitylated, resulting in the recruitment of ubiquitin-binding autophagy receptors, which promote assembly of an autophagosome around damaged lysosomes for delivery to healthy lysosomes for degradation. Here, we review the current state of our understanding of mechanisms used to mark and eliminate damaged lysosomes, and discuss the complexities of galectin function and ubiquitin-chain linkage types. Finally, we discuss the limitations of available data and challenges with the goal of understanding the mechanistic basis of key steps in lysophagic flux.

Introduction:

Lysosomes are the degradative endpoints within eukaryotic cells, but also function as complex signaling organelles linking the recycling of cellular building blocks to a myriad of metabolic pathways. Acidic hydrolases within the lysosome degrade diverse macromolecular substrates derived from cellular and extracellular compartments. These substrates are delivered to the lysosome primarily through the endocytic pathway wherein endosomes containing plasma membrane-derived proteins and other substrates fuse with lysosomes. In addition, damaged or surplus organelles and proteins are recycled via autophagy. These components are surrounded by a double membrane structure called an autophagosome, which subsequently fuses with a lysosome, allowing the contents to be degraded within the lysosomal lumen. Thus, the lysosome constitutes a central cellular hub maintaining protein and organelle homeostasis.

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CONFLICT OF INTERESTS

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Reactive oxygen species or lipid metabolites can permeabilize the lysosomal limiting membrane that results in the leakage of hydrolases into the cytosol triggering cell death. As such, the maintenance of lysosome integrity is indispensable for cellular health. Upon damage, a sequence of endo-lysosomal damage response pathways are activated, resulting in either the repair of partially permeabilized membranes via re-sealing or the degradation of unrepairable lysosomes [1]. ESCRT III-mediated membrane resealing systems provide an acute response [2,3], but if membrane damage persists, such lysosomes are sequestered within autophagosomes, which subsequently fuse with healthy lysosomes in a selective autophagy pathway termed “lysophagy” [4,5]. Understanding the degree of damage required and subsequent signals for driving mechanisms that result in lysophagy as opposed to repair is an active area of research.

Lysosomal membrane integrity is lost in a variety of disease states and stress conditions. Damaged lysosomes are observed in tissues from patients with hyperuricemic nephropathy [6,7] and in tissues of patients with inclusion body myopathy associate with frontotemporal dementia[8]. Oxidative stress, proteases, specific types of lipids, and urate can all result in lysosome membrane permeabilization[9,10]. Damaged lysosomes display: 1) reduced acidity, 2) altered lipid composition, 3) reduced proteolytic capacity, and/or 4) increased propensity for rupture of the limiting lysosomal membrane [11]. Growing evidence indicates that endocytosed neurotoxic aggregates including α-synuclein, Huntington, Aβ, or tau fibrils can promote rupture of endolysosomal membranes, potentially allowing release of toxic aggregation-prone proteins into the cytosol if the damaged organelle isn’t rapidly eliminated [8,12–15]. Despite the many different physiological ways that lysosomes can be ruptured, the majority of studies employ small molecule lysosomotropic agents such as LLOMe (L-leucyl-L-leucine methyl ester) or GPN (glycyl-l-phenylalanine 2-naphthylamide) that promote largely synchronous rupture of lysosomal membranes, allowing kinetic and mechanistic dissection of downstream events [11]. LLOMe enters the lysosomal system via endocytosis and forms conjugates that can specifically rupture lysosomal membranes on a subset of lysosomes to initiate lysophagy, while GPN promotes lysosomal osmotic swelling and rupture. Membrane rupture can initiate a series of steps that facilitate lysophagy: 1) damage sensing by galectins, 2) amplification of the damage signal via ubiquitin conjugation onto lysosome-associated proteins, and 3) ubiquitin-dependent recruitment of autophagy machinery for subsequent capture and elimination of irreversibly damaged lysosomes [16, 17] (Figure 1). Elements within the lysophagy pathway have parallels with, and were in some cases initially discovered in the context of xenophagy, where bacteria-containing damaged vacuoles or phagosomes are targeted and degraded via autophagy [18,19]. Here we describe our mechanistic understanding of the aforementioned steps of lysophagy and elaborate on several areas where gaps in our understanding continue to exist.

Galectin recruitment signals lysosomal membrane rupture

Macromolecules within the lysosomal lumen are modified with glycans, which become exposed upon rupture of the limiting membrane. Specific cytosolic galectins serve a surveillance function and rapidly access the lumen of ruptured lysosomes, where they bind glycans (preferentially beta-galactosides) using a conserved carbohydrate recognition domain (CRD). The galectin family of proteins, with 12 members in humans, conform

to three general prototypes with distinct glycan specificities: homodimeric CDRs, tandem repeat CDRs typically binding unique glycans, and a chimera containing a CDR fused with a domain that interacts with other proteins (Figure 2). Interestingly, unique galectin types are selective to which glycan modifications they will bind [20], and galectins also display distinct patterns of expression across cell types and tissues [20–22].

Four Galectins (LGALS1, LGALS3, LGALS8, and LGALS9) are rapidly recruited to ruptured lysosomes and initiate the process of lysophagy [23–28] (Figure 1,2). However, to date, removal of no single galectin or combination of galectins, has been shown to completely block lysophagic flux, although defects in lysosomal ubiquitylation and recruitment of autophagy machinery (ATG13, ATG16L1, LC3B) to membrane-compromised lysosomes have been reported [24–27]. As such, the precise contributions of specific galectins to downstream processes and the extent of redundancy is incompletely understood.

LGALS3 and LGALS8 (Figure 2), while not required for lysosomal ubiquitylation [29], nevertheless contribute to repair and/or recycling of damaged lysosomes but appear to play roles that are independent of lysosomal ubiquitylation [17,25,26,29]. ALIX – a regulator of the ESCRT III membrane resealing complex – is rapidly recruited to damaged lysosomes and this recruitment is largely abolished in cells lacking LGALS3, indicating a role for LGALS3 in membrane resealing [25]. Indeed, cells lacking LGALS3 fail to effectively repair lysosomal membranes, have reduced capability to recycle damaged lysosomes (as indicated by reduced recruitment of LC3B) but instead display increased nuclear TFEB, indicating a prolonged transcriptional upregulation of lysosomal biogenesis genes [25]. LGALS8 recruitment to damaged lysosomes leads to inhibition of mTOR via interactions with the Ragulator-SLC38A9 system [26]. Ragulator is a lysosomal membrane associated complex that associates with a heterodimeric Rag GTPase complex whose GTPase activity is required for mTOR activation in response to amino acids. In the absence of LGALS8, mTOR is not effectively released from damaged lysosomes, as assessed by immunofluorescence [26]. Consistent with this, proteomic analysis of purified lysosomes in response to lysosomal damage revealed rapid loss of mTORC1 complex subunits (mTOR, Raptor, MLST8) and Rag GTPase subunits, reflecting dynamic release from damaged lysosomes [27]. Negative regulation of mTOR via LGALS8 may act synergistically with AMPK activation to promote clearance of damaged lysosomes via autophagy, as indicated by reduced LC3B lipidation upon lysosomal damage in cells lacking LGALS8 [26].

While cells lacking LGALS3 or LGALS8 display wild-type levels of lysosomal ubiquitylation (ubiquitin puncta as detected by immunofluorescence), deletion of LGALS9 results in a 50–60% reduction in lysosomal ubiquitylation, which is rescued by expression of wild-type LGALS9 but not a glycan binding mutant [29]. Whether this partial reduction in lysosomal ubiquitylation reflects compensatory functions for other galectins is unknown. Interestingly, LGALS9 can associate with the USP9X deubiquitylating enzyme (DUB) (Figure 2), an interaction that is lost upon lysosomal damage, and depletion of USP9X in cells lacking LGALS9 results in rescue of lysosomal ubiquitylation [29] (Figure 3). This finding suggests that LGALS9 itself is not essential for recruitment of ubiquitylation machinery to damaged lysosomes, but may indirectly control access of USP9X to

ubiquitylated lysosomal proteins. LGALS9 also appears to regulate additional signaling systems on lysosomal membranes by displacing USP9X from its associated TAK1 subunit, which in turn activates adenosine monophosphate (AMP)-activated protein kinase (AMPK) and ULK1 phosphorylation to activate autophagy [29] (Figure 3).

Lysosomal ubiquitylation in response to membrane rupture

One of the earliest responses to lysosomal membrane damage is the accumulation of ubiquitin on lysosomes [8,16] (Figure 1). Indeed, pharmacological inhibition of the ubiquitin-activating enzyme (UBA1) completely blocks lysophagic flux in response to damage [27]. In HeLa cells, lysosomal membrane damage is associated with rapid accumulation of K63-linked Ub chains and delayed assembly of K48-linked chains on a subset of damaged lysosomes [8] (Figure 3). Whether these ubiquitylation patterns based on immunostaining reflect distinct states, for example reflecting the extent of initial damage, or simply the maturation state of the process, is currently unclear. Lysosomal ubiquitylation promotes the recruitment of ubiquitin-binding autophagy receptors as described below. During lysosomal ubiquitylation, the p97/VCP AAA^{+}-ATPase – a ubiquitin-dependent segregase – is recruited to a subset of ubiquitylated lysosomes and pharmacological inhibition or genetic depletion of p97 function blocks lysophagy [8] (Figure 3). p97 employs a number of accessory factors to regulate its targets and in the case of lysophagy, the PLAA adaptor protein and YOD1 deubiquitylating enzyme are thought to be associated with p97 on damaged lysosomes. Interestingly, a Ub-binding site on YOD1 is necessary for recruitment to K48-linked conjugates, leading to the idea that YOD1 serves as a sensor for this form of damaged lysosomes [8]. Indeed, current models posit that p97 functions to extract K48-linked ubiquitylated target proteins from the damaged lysosomal membrane which precedes it’s clearance [16] (Figure 3). This hypothesis is supported by the findings that in the presence of catalytically inactive p97, K48-linked conjugates and LC3B accumulate on damaged lysosomes that are not effectively cleared [8]. Interestingly, subsequent proteasomal degradation of the K48-Ub modified proteins is not required, as proteasome inhibition does not block lysophagy [8]. Nevertheless, the underlying biochemical mechanisms are yet to be established and precisely how this step could be required for “licensing” autophagy remains unclear, especially given the apparent role of K63-Ub chains in recruiting autophagy adaptors as described below.

While ubiquitylation is essential for lysophagic flux, our understanding of this process is fragmentary. Active areas of research include 1) identifying lysosomal proteins or possibly non-proteinaceous components that are ubiquitylated in response to membrane rupture 2) identifying relevant ubiquitylation machinery, and 3) understanding how relevant machinery is either directed to damaged lysosomes or activated upon loss of lysosomal membrane integrity. As mentioned above, LGALS9 deletion reduces lysosomal ubiquitylation upon damage but this effect is eliminated upon co-depletion of USP9X, indicating that LGALS9 is not an essential gate-keeper for lysosomal ubiquitylation [29]. LGALS3 has been linked with recruitment of the TRIM16 ubiquitin ligase to damaged lysosomes, but in this context, TRIM16 appears to associate with ULK1, Beclin and ATG16 and to ubiquitylate Beclin1, but has not been shown to directly ubiquitylate components of the lysosome itself [24] (Figure 3). Indeed, cells genetically engineered to lack LGALS3 have no apparent defect

in lysosomal ubiquitylation as assessed using immunofluorescence [25]. Thus, the role for TRIM16 as Ub ligase directly regulating lysophagy through ubiquitylation of lysosomally localized proteins remains unclear.

The primary candidate E2 and E3 enzymes for ubiquitylation of damaged lysosomes are the E2 conjugating enzyme UBE2QL1, and two E3 ligases - SCF^{FBXO27}, and SCF^{FBXO2}[30–32] (Figure 3). However, whether each of these components function independently or in concert is unclear, and there is no evidence that UBE2QL1 can function in the context of an SCF ubiquitin ligase. UBE2QL1 accumulates on damaged lysosomes with kinetics that correlate with assembly of K48-linked chains (~1–2 hours), and depletion of UBE2QL1 by siRNA partially reduces both the recruitment of p97 and the clearance of LGALS3-positive lysosomes but does not eliminate K63 ubiquitylation [30] (Figure 3). Therefore, while UBE2QL1 appears to play an important modulatory role, the E3 ligase(s) that function together with this E2 in this context are unknown.

SCF^{FBXO27} and SCF^{FBXO2} are composed of the scaffold protein CUL1, the RING domain protein RBX1, and the SKP1-FBXO27 (or FBXO2) substrate adaptor module, and the activity of SCF complexes requires that CUL1 be specifically neddylated by the NAE1 NEDD8 activation and transfer machinery [33]. Interestingly, FBXO27 and FBXO2 contain an “F-box associated domain” capable of binding to carbohydrate moieties on proteins [34] and are recruited to damaged lysosomes, within minutes of LGALS3 recruitment in the case of FBXO27 [31,32]. Overexpression of SCF^{FBXO27} promotes K48-linked ubiquitylation of LAMP2 and to a lesser extent LAMP1 [31], but whether this E3 ligase is responsible for assembly of K48-linked chains that are removed by p97-YOD1 is unknown (Figure 3). Deletion of FBXO27 in PANC-1 cells leads to a modest reduction in ubiquitin-binding receptor SQSTM1 and LC3B localization at damaged lysosomes and clearance of LGALS3-positive lysosomes [31]. However, significant lysosome clearance was still present, indicating that SCF^{FBXO27} is not absolutely essential for this process [31]. SCF^{FBXO2} has been studied in the context of a Nieman Pick type C disease model [32]. In NPC1 mutant fibroblasts, unesterified cholesterol accumulates in late endosomes and lysosomes, rendering these organelles more susceptible to membrane damage and recruitment of LGALS3. Overexpressed FBXO2 is recruited to damaged lysosomes in fibroblasts and Fbxo2−/− cortical neurons display a slight delay in turnover of LGALS3 upon lysosomal damage [30]. NPC1 mutant mice additionally deficient in FBXO2 exhibited significantly worse motor function and decreased survival [32], but whether this is a reflection of defects in in lysophagy is unknown. However, the general and absolute requirement for these E3 ligases in lysophagic flux is brought into question by the finding that this process is not blocked by the cullin neddylation inhibitor MLN4924 under conditions that eliminate CUL1 neddylation in HeLa cells (M.J.H., Julia Paoli, J.W.H., unpublished results), but is blocked by inhibition of the ubiquitin E1 enzyme with an analogous small molecule inhibitor MLN7243 both HeLa and fibroblasts [27]. Given that cullin neddylation is required for SCF activation and indeed all cullin-RING E3 ligases [33], it would appear that this broad class of E3 ligases is not required for lysophagic flux in HeLa cells.

Taken together, the available data suggest that further studies are needed to identify additional ubiquitylation machinery responsible for marking damaged lysosomes, and for understanding any cell-type dependent control of the process, for example via distinct E2 and E3 enzymes. In particular, the identification of machinery capable of assembly of K63-linked chains on damaged lysosomes would represent a step forward, given the apparent role of K63-linked Ub chains in recruitment of autophagy receptors, as discussed below.

Role of Ub-binding autophagy receptors in lysophagy

Previous studies have identified a small group of related proteins as key receptors linking cargo ubiquitylation with assembly of autophagosomes *in situ* on the ubiquitylated organelle [35]. These proteins – typified by OTPN (also called optineurin), TAX1BP1, NBR1, CALCOCO2 (also called NDP52), and SQSTM1 (also called p62) contain C-terminal Ub-interacting domains and extensive coiled-coil domains (except for SQSTM1) that typically serve as dimerization domains (Figure 4A). Multiple types of Ub-binding domains are found within these proteins, including UBZ1, UBA, Znf, and UBAN domains [36], and while systematic data is lacking, these domains appear to generally prefer association with linear or K63-linked ubiquitin chains, as measured *in vitro* [37–39], although in some studies CALCOCO2 was found to bind M1, K48, and K63 chains equally well [39]. These Ub-binding domains bind to ubiquitylated cargo to promote autophagosome formation [35]. In addition, these receptors contain short motifs that interact with either ATG8 proteins on the surface of growing phagophores and/or the C-terminal “claw” domain of FIP200 (also called RB1CC1) (Figure 4A–D). Historically, the LC3 interacting region (LIR) was demonstrated to bind directly to ATG8 proteins, but more recently, LIR-like motifs have been shown to also bind to the FIP200 claw (referred to as FIR motifs), thereby recruiting the FIP200-ULK1 kinase to ubiquitylated cargo [40–43] (Figure 4C). In addition, CALCOCO2 and TAX1BP1 contain N-terminal SKICH domains that can independently bind the coiled-coil region of FIP200 (Figure 4B,C) [41,44,45]. It is thought that ULK1 kinase activity in the proximity of the autophagic cargo leads to activation of PI3P kinase activity and WIPI protein recruitment, thereby initiating autophagosome assembly *in situ* (Figure 4B,D). This mechanism explains how autophagosome formation is limited to the target organelle for elimination [46,47].

Early studies using RNAi indicated that SQSTM1 promotes clearance of LGALS3-positive lysosomes [1,8]. However, subsequent studies in HeLa cells indicate that lysosomal damage leads to rapid (<30 min) recruitment of multiple receptors – including OTPN, TAX1BP1, CALCOCO2, and SQSTM1. Using GFP-RFP-LGALS3 or mKeima-LGALS3 reporters to measure lysophagic flux, it was found that deletion of SQSTM1, OPTN, or CALCOCO2 did not block lysophagic flux [27]. In contrast, cells engineered to lack TAX1BP1 (Figure 4B) were profoundly defective in lysophagy, as determined using both flux reporters and galectin puncta clearance assays in HeLa cells or induced neurons [27]. A common feature of these receptors is association with the TBK1 protein kinase, either directly in the case of OPTN or through one of two adaptor proteins (NAP1 and SINTBAD) in the case of SKICH domain receptors (Figure 4B,C), and TBK1 is required for other types of autophagic flux for cargo ranging from intracellular bacteria to mitochondria [39,41,48,49]. Indeed, HeLa cells

or induced neurons either lacking the TBK1 gene or TBK1 activity via pharmacological inhibition also display defects in lysophagic flux [27].

Taken together, the current model for cargo receptor function in lysophagy is that upon membrane rupture and lysosome ubiquitylation, TAX1BP1 in association with TBK1 is recruited to lysosomes (Figure 4B). In this context, TAX1BP1 can recruit the ULK1-FIP200 complex to initiate *in situ* phagophore initiation. Evidence for this model includes the finding that deletion of the SKICH domain, or mutations that render SKICH unable to associate with FIP200, inactivates TAX1BP1-dependent lysophagic flux [27]. As with CALCOCO2 in bacterial autophagy [44], TAX1BP1 can also interact with LGALS8 [50] and could therefore be brought to damaged lysosomes independently of a ubiquitin signal. However, mutation of the C-terminal UBZ1 in TAX1BP1 impairs lysophagic flux [27], indicating that lysosomal ubiquitylation plays a role in amplifying autophagic signaling. In contrast, mutation of sequences in TAX1BP1 that bind LGALS8 do not impact lysophagic flux [27]. Interestingly, in HeLa cells lacking OPTN, CALCOCO2, and TAX1BP1, ectopic expression of OPTN – but not CALCOCO2 – can rescue lysophagic flux and this activity is absolutely dependent on the ability of OPTN to bind ubiquitin [27].

Open questions in lysophagy

Data accumulated thus far indicates a complex interplay between galectins, membrane repair machinery, the ubiquitin system, and autophagy machinery in the cellular decisions as to the fate of damaged lysosomes, but several aspects remain mechanistically unresolved. Central to this is the decision of whether to repair damaged lysosomal membranes via ESCRT-related pathways. Although ESCRT proteins are known to be rapidly recruited to damaged lysosomes[2,3,25,27,51], what determines the damage threshold for repair versus degradation is unclear, as are any mechanisms that control “switching” between the two pathways [17]. Analysis of the earliest stages of lysosomal damage has primarily relied on clearance of LGALS3-positive puncta using immunofluorescence [28], which provided an indirect readout of the process. The development of tandem GFP-RFP or mKeima lysosome flux assays allows for quantitative analysis of the delivery of damaged lysosome to healthy lysosomes for degradation via a change in fluorescent property upon autophagosome-lysosome fusion but not complete loss of fluorescence [7,11,27], Such assays should now be used to facilitate a more rigorous analysis of the pathway. Among the most pressing questions concerns the identity of the enzymatic machinery necessary for K63-linkage ubiquitylation of damaged lysosomes. As described above, the E3 ligases responsible for tagging lysosomes, as well as the relevant targets within the organelle, are not clearly defined (Figure 3). The finding that cells depleted of UBE2QL1 maintain significant K63-linked chains [30] indicates that additional E2s are likely to be involved, and the expectation is that such E2s would function together with relevant E3 ligases to produce K63-linked chains. We note that lysosomes harbor several resident RING-domain containing E3 ligases including RNF13, RNF167, and RNF152, although none of these has been linked with lysophagy. In the case of bacterial autophagy, two Ub ligases – RNF213 and LUBAC (linear Ub assembly complex) – have been demonstrated to work in sequence to ubiquitylate both the LPS molecule on the bacterial surface as well as proteins on the bacteria or vacuole membrane [52–54]. Both of these enzymes are capable of linking Ub to non-proteinaceous

moieties via oxyester linkages [52,55] and it is conceivable that glycans present within the lysosomal membrane could also be modified by relevant lysophagy E3 ligases rather than canonical lysine residues in proteins. The ability to elucidate the extent to which mechanisms of lysophagy are cell type specific, and the machinery responsible for pathway activation, will require identification of relevant E3 ligases across a range of cell types and damaging agents. Finally, the identification of the relevant enzymes is critical for providing mechanistic clarity to the underlying process, as well as eventual goals of reconstituting key steps in the pathway [46]. This includes a determination of the lysosome-associated molecules that are directly conjugated with ubiquitin as a first step in understanding whether target specificity plays a role in the ultimate recruitment of the ubiquitylation machinery.

Also unclear is the extent to which galectins themselves support recruitment of the ubiquitylation machinery. Although deletion of LGALS9 reduces lysosomal ubiquitylation, this activity is completely rescued upon co-depletion of USP9X [29]. As such, no single galectin has been demonstrated to be required for ubiquitylation of damaged lysosomes. Systematic genetic analysis of galectin mutants may be required to understand relevant dependences once relevant ubiquitylation machinery is identified. The development of a more comprehensive understanding of the molecular mechanisms involved will likely facilitate future studies aimed at understanding the identity of endogenous triggers of lysosomal damage, and how pathogenic states may contribute to lysosomal dysfunction.

ACKNOWLEDGMENTS

J.W.H. acknowledges research support from the NIH (AG011085, NS083524, NS110395, U24 HG006673), Aligning Science Across Parkinson's (ASAP), Chan-Zuckerberg Neurodegeneration Challenge Network Collaborative Grant, and the Bluefield Project. The Michael J. Fox Foundation administers the grant ASAP-000282 on behalf of ASAP and itself. For the purpose of open access, the author has applied a CC-BY public copyright license to the Author Accepted Manuscript (AAM) version arising from this submission. M.J.H. was supported by a postdoctoral fellowship from Jane Coffin Childs Foundation.

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complex does not get properly recruited to damaged lysosomes when cells lack LGALS3. Additionally, LGALS3 knockout cells fail to effectively repair lysosomal membranes and display persistent nuclear TFEB, indicating a prolonged transcriptional response for lysosomal biogenesis. Autophagy component puncta formation was diminished in LGALS3 knockout cells. At later lysosome damage time points LGALS3 interacts with TRIM16, a scaffold protein that potentially promotes autophagic clearance of lysosomes.

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untreated cells, depletion of UBE2QL1 results in increased lysosomal damage, mTOR dissociation from lysosomes (signals inactivation of mTOR and subsequent upregulation of autophagy), and TFEB activation (TFEB translocates to the nucleus to upregulate lysosomal biogenies).

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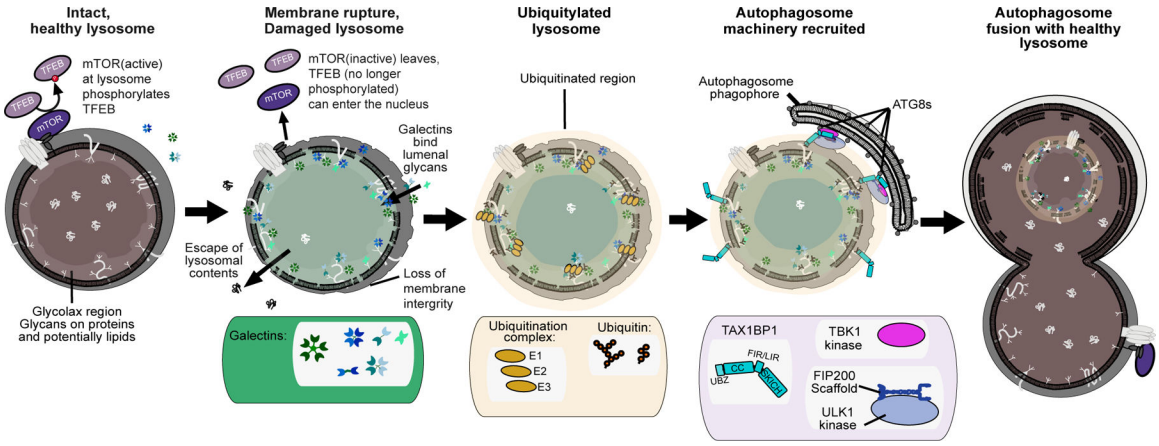

Figure 1.

Overview of lysophagy. Healthy lysosomes have an intact membrane with an acidified lumen. Upon rupture of the limiting membrane, cytosolic galectins get access into the lumen where they associate with glycans. Rupture also results in the recruitment of ubiquitin ligase machinery which promotes the ultimate recruitment of autophagy receptors, thereby nucleating assembly of an autophagosome around the damaged lysosome. The autophagosome ultimately fuses with a healthy lysosome, thereby allowing recycling of the materials present in the damaged lysosome.

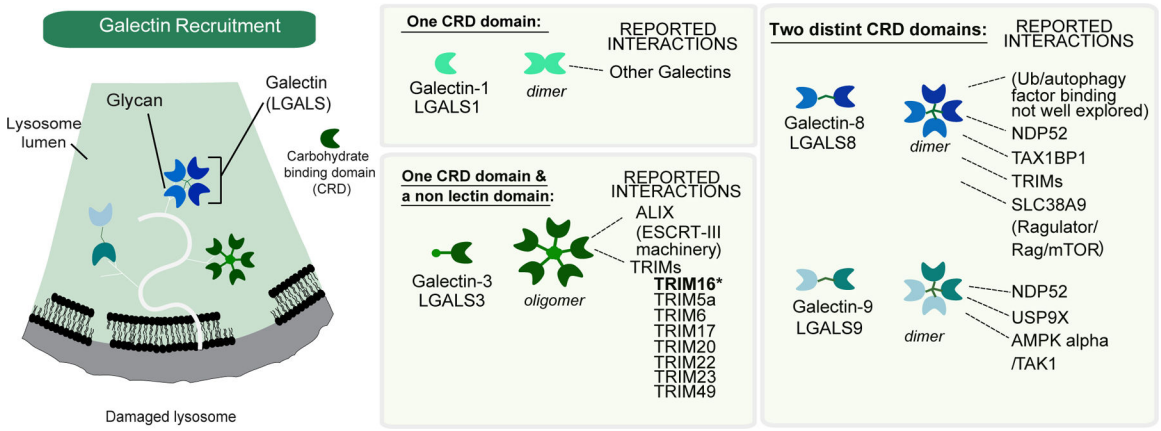

Figure 2.

Overview of galectins. Galectins are rapidly recruited to the lumen of lysosomes upon membrane rupture, where they associate with glycans. Multiple classes of galectins have been shown to be recruited to damaged lysosomes, including LGALS3, LGALS8, and LGALS9. Galectins associate with glycans through their carbohydrate recognition domains (CRD). While some galectins have single CRDs, others have two CRD domains in a single polypeptide while others oligomerize to create a cluster of CRDs. Various galectins have been reported to associate with multiple classes of proteins, ranging from autophagy receptors and components of the membrane repair apparatus to members of the TRIM family of ubiquitin ligases.

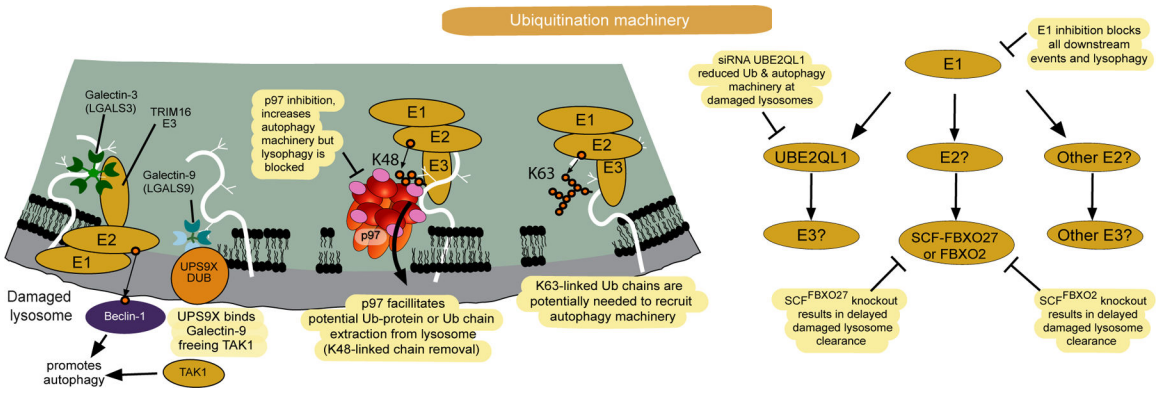

Figure 3.

Lysosome ubiquitylation in response to membrane rupture. In response to membrane rupture, lysosomal proteins are ubiquitylated thereby promoting recruitment of autophagy receptors (left panel). Multiple E2 and E3 enzymes have been reported to function in lysosomal ubiquitylation or ubiquitylation of autophagy machinery, including the E2 enzyme UBE2QL1, and the E3 ligases SCF^{FBXO27} and TRIM16 (left and right panels). TRIM16, which is recruited via LGALS3, has been reported to ubiquitylate the Beclin subunit of the VPS34 PI3 kinase complex. SCF^{FBXO27} is reported to ubiquitylate LAMP1 and LAMP2 in response to membrane rupture but cells lacking FBXO27 have only a modest defect in clearance. p97 promotes extraction of proteins labeled with K48-chains likely downstream of the UBE2QL1 E2 enzyme.

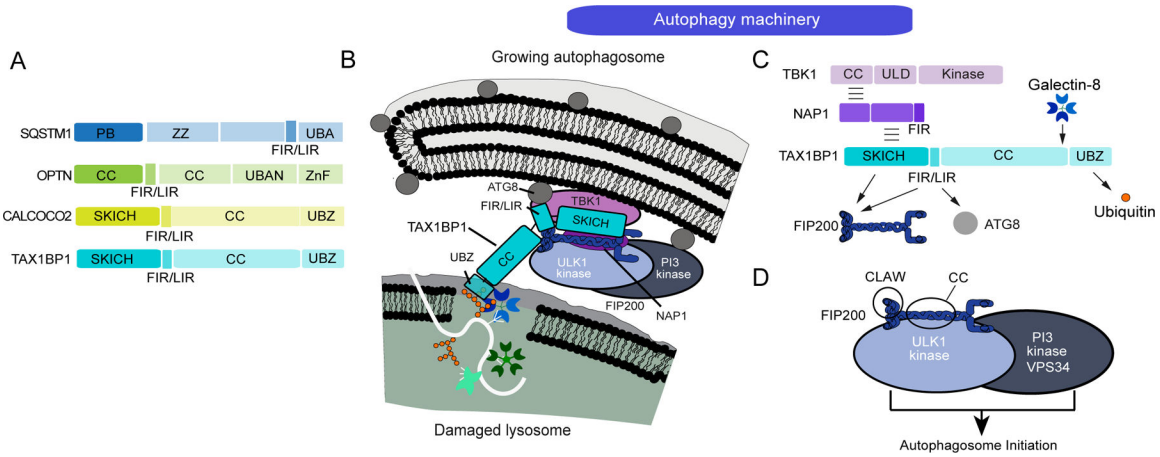

Figure 4.

Recognition of ubiquitylated lysosomes by the autophagy receptor TAX1BP1. (A) Domain organization for ubiquitin-binding autophagy receptors. (B) Model depicting molecular interactions between damaged lysosomes and the autophagosome initiation machinery. TAX1BP1 interacts with the FIP200-ULK1 complex, which in turn interacts with the VPS34 PI3 kinase complex. (C) Schematic showing interactions between TAX1BP1 and the autophagy machinery. TAX1BP1 contains an N-terminal SKICH domain and FIR motifs that both associate with the FIP200 claw domain and sequences within the coiled-coil. The SKICH domain also associates with the TBK1 kinase complex via the adaptor proteins NAP1 and SINTBAD. The C-terminal UBZ domain binds ubiquitin chains. (D) Schematic of the FIP200-ULK1 complex and its association with the VPS34 PI3 kinase complex, whose recruitment to autophagic cargo can initiate autophagosome formation via formation of PI3P on target membranes.