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Review

Golgi-Associated ATG8ylation: Mechanisms and Biological Implications

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Abstract: ATG8ylation, the covalent conjugation of LC3/GABARAP-family proteins to lipids or proteins, is no longer viewed solely as a signature of canonical degradative autophagy. A rapidly growing literature has revealed that ATG8 conjugation also occurs on pre-existing single membranes through noncanonical autophagy, a process also referred to as conjugation of ATG8 to single membranes (CASM). The Golgi apparatus has emerged as a particularly important and conceptually revealing platform for this biological process. Recent studies have established that Golgi-associated ATG8ylation is not a passive by-product of organelle disruption, but a regulated response that can be initiated at the Golgi, often depends on the V-ATPase-ATG16L1 machinery, promotes stress-responsive transcriptional programs involving TFE3 and TFEB, mediates unconventional secretion of IL1β, and supports the repair of damaged Golgi architecture. Here, we synthesize these advances into a unified model in which Golgi-associated ATG8ylation functions as a membrane stress-response hub. In this model, Golgi perturbations are sensed through local changes in organelle state, including trafficking blockade, pH/ion imbalance, V-ATPase assembly, and cytoskeletal or ribbon disruption. These signals recruit or activate ATG8 conjugation machinery on Golgi membranes and generate functional outputs: membrane remodeling, Golgi repair, transcriptional stress adaptation, and unconventional secretion. We also discuss unresolved questions concerning membrane specificity, lipid acceptors, the distinction between repair and degradation, and the physiological roles of this pathway.

Keywords: ATG16L1; ATG8ylation; CASM; Golgi apparatus; Golgi repair; IL1β; LC3; noncanonical autophagy; TFE3; TFEB; trans-Golgi network; unconventional secretion; V-ATPase

1. Introduction

Macroautophagy is classically defined as a lysosome-directed degradative process in which cytoplasmic material is captured within double-membrane autophagosomes [1]. The core autophagy genes encode a conserved machinery for autophagosome initiation, nucleation, expansion, closure, and fusion with lysosomes [2]. A central feature of this pathway is the conjugation of ATG8-family proteins to membrane lipids, a reaction first established through studies of yeast Atg8 and mammalian LC3 [3,4]. In canonical autophagy, ATG8 lipidation depends on ubiquitin-like conjugation systems involving ATG7, ATG3, ATG12-ATG5, and ATG16L1, and it is spatially coordinated by upstream autophagy factors including the ULK1/2-ATG13-FIP200-ATG101 complex, class III phosphatidylinositol 3-kinase complex I (PI3KC3-C1) and WIPI proteins, which together initiate and organize the nascent phagophore [5,6].

This canonical view has been revised by the discovery that LC3 and GABARAP proteins can be conjugated to pre-existing single membranes in settings that do not generate autophagosomes. LC3-associated phagocytosis (LAP), LC3-associated endocytosis (LANDO), entosis-associated macroendocytic processing, and LC3-associated macropinocytosis all illustrated that ATG8 lipidation can be deployed at membranes that are not double-membrane autophagosomes [7–11]. Since lipidated LC3 is widely used as a marker for autophagosomes, careful interpretation of LC3 signals remains essential, particularly when perturbations affect lysosomal acidification, autophagosome maturation, or noncanonical ATG8 conjugation pathways [12]. Noncanonical ATG8ylation retains the core ATG8 conjugation machinery but bypasses several canonical initiation factors such as ULK1, ATG13 and FIP200 [13]. Endolysosomal osmotic or ionic perturbations further revealed that lysosome-related single membranes can recruit LC3 through vacuolar-type H^+-ATPase（V-ATPase)-linked mechanisms [13,14]. The term conjugation of ATG8 to single membranes (CASM) has been adopted to describe these conjugation events on single membranes, and lipidomics showed that CASM can conjugate ATG8 proteins not only to phosphatidylethanolamine but also to phosphatidylserine [13,15,16]. The field now increasingly uses ATG8ylation as an umbrella term for membrane and protein conjugation events involving ATG8-family proteins [16–19].

Although the Golgi has long been recognized as a trafficking and stress-signaling hub, only more recently has it become clear that Golgi membranes can themselves act as platforms for noncanonical ATG8 lipidation. This is conceptually important because the Golgi is not merely an intermediate station in biosynthetic traffic. It is a polarized, highly dynamic, and stress-responsive organelle that integrates membrane trafficking, glycosylation, lipid metabolism, organelle positioning, cytoskeletal organization, and secretion [20,21]. These considerations provide the rationale for reviewing Golgi-associated ATG8ylation as a distinct and rapidly developing area of noncanonical autophagy research. Here, we collect the evidence that Golgi membranes serve as platforms for ATG8 lipidation, summarize the mechanisms that connect this process to the V-ATPase-ATG16L1 axis, and discuss its potential roles in stress signaling, unconventional secretion, membrane remodeling, and Golgi repair.

2. From Canonical Autophagy to CASM at Golgi Membranes

The main distinction between canonical autophagy and CASM is not the identity of the downstream conjugation enzymes, but the membrane context and recruitment logic that bring those enzymes to the site of lipidation. In canonical autophagy, upstream autophagy factors mentioned above, including the ULK1 complex, PI3KC3-C1 and WIPI proteins, coordinate the growth of a specialized isolation membrane. The ATG12-ATG5-ATG16L1 complex then defines the site at which LC3/GABARAP proteins are lipidated [5,6]. CASM, in contrast, directs the same core conjugation systems to pre-existing membranes such as phagosomes, endolysosomes, macropinosomes, pathogen-containing vacuoles, and, as now recognized, Golgi membranes [7–10,13–18,22–31].

A defining molecular feature of many CASM pathways is the requirement for the C-terminal WD40 domain of ATG16L1 [13,32]. The WD40 domain is dispensable for canonical autophagy but is required for noncanonical LC3 lipidation at single membranes [33]. Mechanistically, the WD40 domain mediates the inducible interaction of ATG16L1 with V-ATPase, providing a basis for the selective recruitment of the ATG16L1 complex to CASM membranes [16]. This mechanism, termed V-ATPase-ATG16L1-induced LC3 lipidation (VAIL), helps explain the specificity of CASM. V-ATPase is a multisubunit proton pump composed of a peripheral V1 subcomplex, which hydrolyzes ATP, and a membrane-embedded V0 subcomplex, which mediates proton translocation [34,35]. The V1 and V0 subcomplexes can reversibly assemble and disassemble, allowing dynamic regulation of V-ATPase activity in response to changes in cellular and organelle conditions [35]. Following dissipation of the pH gradient, assembly of the V1-V0 increases, accompanied by enhanced recruitment of ATG16L1. This is thought to occur because the fully assembled V-ATPase adopts a conformation that permits ATG16L1 binding, whereas the dissociated V1 and V0 do not efficiently support this interaction [16,36]. Bacterial effector SopF that modify the V-ATPase can prevent recruitment of ATG16L1 and block xenophagy-associated lipidation [37]. STING-induced LC3B lipidation onto single-membrane vesicles likewise depends on V-ATPase and ATG16L1-WD40 interactions [38]. A broader analysis of LAP and other types of CASM further supports V-ATPase as a universal regulator of multiple single-membrane ATG8ylation responses [39].

The Golgi apparatus is a polarized organelle composed of cis-, medial-, and trans-Golgi cisternae, with the trans-Golgi network (TGN) serving as a major sorting station for post-Golgi trafficking [20,40]. Beyond its classical roles in protein glycosylation, lipid sorting, and cargo transport, the Golgi is increasingly recognized as a stress-responsive signaling platform [41]. Golgi luminal acidity increases progressively along the secretory pathway and is maintained in part by organelle-localized V-ATPase complexes [21]. Notably, this basal proton-pumping function should be distinguished from stress-associated changes in V0-V1 assembly or V-ATPase activity.

Golgi-associated ATG8ylation therefore fits within the broader CASM framework while raising additional questions related to membrane identity and spatial organization. Several Golgi-directed perturbations induce ATG8 lipidation in a V-ATPase- and ATG16L1-dependent manner [23,25,27–30], yet how Golgi membrane identity is coupled to ATG16L1 recruitment remains incompletely understood. Moreover, because the Golgi is highly polarized, LC3 recruitment to the TGN, cis/medial Golgi compartments, ERGIC-associated membranes, or post-Golgi vesicles may represent mechanistically related but spatially distinct forms of ATG8ylation. Importantly, Golgi-associated CASM should also be distinguished from canonical Golgiphagy. Golgiphagy relies on ATG8-binding receptors such as CALCOCO1, YIPF3/YIPF4, and TM9SF3 to target Golgi material for degradation [42–45]. By contrast, Golgi-associated CASM is best viewed as a stress-responsive, single-membrane ATG8ylation pathway whose immediate outputs may include signaling, sorting, secretion, and membrane restoration rather than lysosomal degradation.

3. The Emergence of Golgi-Associated ATG8ylation

The first major line of evidence came from pharmacological studies using AMDE-1 or unsaturated fatty acids, complemented by genetic analyses, which showed that Golgi-associated LC3 lipidation can be induced independently of canonical autophagy and requires V-ATPase [23,26]. These experiments were important because they separated LC3 lipidation from starvation-induced autophagosome formation and suggested that the Golgi complex itself could provide a membrane platform for CASM. Silva and colleagues demonstrated LC3 recruitment to damaged Golgi [24]. Liu and colleagues also identified niclosamide as an inducer of noncanonical LC3 lipidation on Golgi [25]. Cerrato and colleagues later showed that oleate causes LC3 accumulation at the TGN and linked this phenomenon to blockade of protein trafficking [27]. Later, another study found that overexpression of Rab2B causes structural changes in the Golgi and induces LC3 aggregation at the ERGIC and cis-Golgi [31], which also disrupts conventional protein secretion suggesting that the relevant trigger may often be impaired Golgi exit rather than general autophagy activation. ATG8ylation on secretory membranes suggested that LC3 lipidation can occur on membranes closely related to the ERGIC-Golgi secretory axis [46]. These observations helped frame Golgi-associated ATG8ylation not as an isolated phenomenon but as part of a wider family of stress-responsive single-membrane ATG8 conjugation events.

4. Molecular Machinery and Membrane Logic

Recent work has moved the field from phenomenology to mechanism, with the decisive advance coming from the direct linkage of Golgi-associated LC3 lipidation to the CASM core machinery. Kang and colleagues demonstrated that Delta-like non-canonical Notch ligand 1 (DLK1) overexpression, as well as Golgi-damaging reagents, induces Golgi-LC3 lipidation in an ATG12-ATG5-ATG16L1-dependent manner [28]. They further showed that post-Golgi trafficking blockade is the primary upstream event and that ATG16L1 recruitment depends on its interaction with V-ATPase through the WD40 domain [28]. This moved the field beyond descriptive localization and placed Golgi-associated ATG8ylation squarely within the VAIL logic that defines CASM more generally [13,33,37–39].

Long and colleagues extended this framework and found that the TGN is a major platform for Golgi-associated ATG8ylation [29]. In their study, compounds including AMDE-1, hexachlorophene, salifungin, and nitazoxanide induce LC3 lipidation on TGN membranes rather than lysosomes [29]. These LC3-positive structures do not behave as canonical degradative autophagosomes: they do not efficiently fuse with lysosomes and they do not promote long-lived protein degradation. This pathway also requires VAIL and is associated with drug-induced elevation of the luminal pH of the TGN, together with increased recruitment of the cytosolic V1 complex to the TGN and enhanced V1-V0 assembly [29].

These data collectively support a working model in which Golgi-associated ATG8ylation is triggered when Golgi membranes experience stress associated with altered luminal chemistry, post-Golgi export blockade, or membrane injury. Conventional secretion depends on ER-to-Golgi and intra-Golgi transport, cargo sorting, and TGN exit [46]. In the context of secretory traffic, tools such as the RUSH system have made it possible to synchronize secretory cargo movement and distinguish trafficking blockade from generalized organelle stress [47]. Classical Golgi-disrupting agents, including brefeldin A, nocodazole, and related compounds, perturb distinct aspects of Golgi organization or trafficking and can induce Golgi-associated CASM [30,48]. Notably, brefeldin A can also suppress Golgi-associated CASM induced by other compounds, likely by dispersing the Golgi membrane platform required for LC3 lipidation [23,25,29]. Their ability to induce or modify Golgi-associated ATG8ylation suggests that the pathway may monitor several convergent features of Golgi dysfunction: failed post-Golgi exit, disrupted Golgi pH, loss of ribbon organization, and altered cytoskeletal anchoring (Table 1).

Table 1. Representative Golgi-associated ATG8ylation models and their upstream features.

Perturbation	Predominant Reported Compartment	Predominant Upstream Feature
Oleate, fendiline, sertraline, and other oleate mimetics [27]	TGN	Protein trafficking blockade
AMDE-1, hexachlorophene, salifungin, and nitazoxanide [23,29,49]	TGN	TGN luminal pH elevation, increased V0-V1 assembly
Niclosamide [25,28,50]	TGN	Protein trafficking blockade, increased V0-V1 assembly
DLK1 overexpression [28]	TGN	Protein trafficking blockade, TGN luminal pH elevation
Rab2B overexpression [31]	ERGIC and cis-Golgi	Protein trafficking blockade, increased V0-V1 assembly
TRIM46 loss [30]	Fragmented Golgi/TGN	Cytoskeletal or Golgi ribbon disruption
Nocodazole/vinblastine [30]	Fragmented Golgi/TGN	Cytoskeletal or Golgi ribbon disruption
Brefeldin A [23,25,29,30]	Fragmented Golgi/TGN	Golgi architecture and trafficking disruption (Note: Brefeldin A can also suppress compound-induced Golgi CASM by dispersing the membrane platform)
Local laser damage, ManII-HRP/DAB, redaporfin-PDT [24]	Golgi stack	Direct Golgi damage

Under such conditions, the V-ATPase assembly state and ATG16L1 recruitment appear to serve as key upstream determinants of LC3 conjugation. At the Golgi, V-ATPase maintains basal luminal acidity but can also participate in stress-induced ATG8ylation through changes in V0-V1 assembly. Importantly, however, Golgi-associated CASM does not appear to be uniformly driven by a pH-dependent VAIL mechanism: activation of the V-ATPase-ATG16L1 axis can occur without detectable Golgi pH elevation, and TRIM46 deficiency induces V-ATPase-dependent but SopF-insensitive ATG8ylation [28,30]. Thus, distinct V-ATPase-dependent mechanisms may operate at the Golgi.

Beyond the VAIL pathway, another recently described CASM mechanism is sphingomyelin and tectonin beta-propeller repeat containing 1 (TECPR1)-induced LC3 lipidation [16,51,52]. In this pathway, TECPR1 replaces ATG16L1 in the ATG12-ATG5 E3-like complex and recognizes cytosolically exposed sphingomyelin on damaged membranes. Whether selected forms of Golgi damage can also engage this alternative E3-like system remains unknown. At present, the strongest direct evidence for Golgi-associated ATG8ylation supports the ATG12-ATG5-ATG16L1 route [28–30].

Together, these studies established that Golgi-associated LC3 lipidation is not restricted to a single drug or damage paradigm, but can arise in response to mechanistically diverse perturbations that converge on Golgi membrane homeostasis (Figure 1).

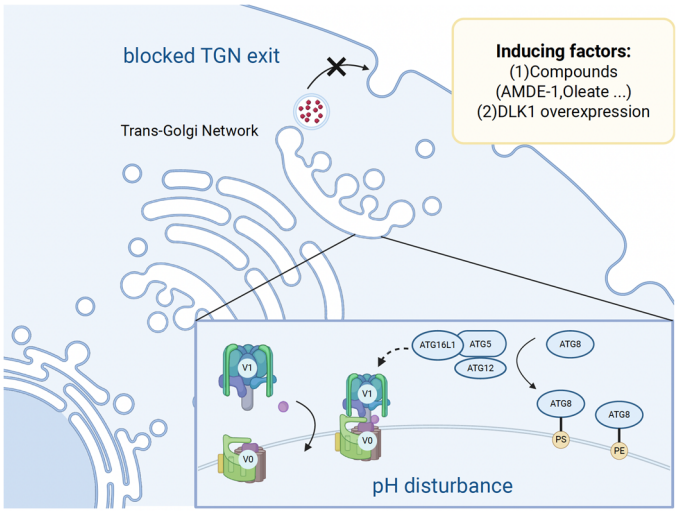

Figure 1. Mechanistic model of TGN-associated CASM triggered by TGN exit blockade or pH disturbance. Multiple stimuli including compounds and gene alterations (DLK1 overexpression) can initiate TGN-associated CASM. Blocked vesicle exit and luminal pH disturbance promote the assembly of the V-ATPase (V1-V0 complex) on the TGN membrane. Assembled V-ATPase recruits the ATG12-ATG5-ATG16L1 complex, which drives ATG8

lipidation with phosphatidylserine (PS) or phosphatidylethanolamine (PE). PS and PE are depicted as illustrative lipid acceptors in this figure, this lipid-conjugation mode for TGN-associated CASM remains experimentally unproven. This cascade illustrates how TGN stress initiates noncanonical ATG8ylation, independent of canonical autophagy regulators. Figure was created in BioRender.com.

5. Functional Outputs of Golgi-Associated ATG8ylation

Golgi-associated ATG8ylation has rapidly evolved from a descriptive marker of organelle perturbation into a mechanistically defined pathway with distinct functional consequences (Figure 2). One important advance came from studies linking Golgi-associated LC3 lipidation to transcriptional stress signaling [28,30]. Kang and colleagues showed that loss of ATG16L1, which impairs Golgi-associated LC3 lipidation, reduces the nuclear translocation of transcription factor binding to IGHM enhancer 3 (TFE3) after inhibition of post-Golgi trafficking, thereby attenuating the Golgi stress response [28]. It highlights Golgi-associated ATG8ylation as a stress-responsive mechanism that connects Golgi perturbation with adaptive transcriptional programs [28,41,53].

Golgi-associated ATG8ylation has also been connected to unconventional secretion [29]. Long and colleagues found that chemically induced TGN-associated noncanonical LC3 lipidation promotes Interleukin-1 beta (IL1β) secretion, whereas canonical autophagy induction suppresses it [29]. This effect was observed not only in LPS-primed THP-1 cells but also in non-immune cells expressing mature IL1β, indicating that TGN-associated ATG8ylation can support IL1β release across distinct cellular contexts [29]. Notably, the authors concluded that this route enhances IL1β secretion independently of gasdermin-mediated pore formation, suggesting that TGN-associated LC3-positive membranes may provide an unconventional trafficking route for mature IL1β [29].

More recently, Golgi-associated ATG8ylation has been implicated directly in organelle maintenance and repair. In the now-published study by Oh and colleagues, TRIM46 deficiency caused microtubule disorganization and Golgi ribbon fragmentation, accompanied by transcription factor EB (TFEB) activation, increased lysosomal biogenesis, and accumulation of LC3B and GABARAP on TGN membranes [30]. This TGN-associated ATG8ylation was nondegradative and mechanistically CASM-like, being largely independent of upstream canonical autophagy regulators but dependent on the core ATG8ylation machinery [30]. Importantly, siRNA-mediated depletion of ATG7 substantially reduced TFEB nuclear localization in TRIM46-deficient cells, linking CASM to TFEB activation and the associated lysosomal biogenesis [30]. In parallel, depletion of ATG4B, ATG5, or ATG7 further exacerbated Golgi fragmentation, indicating that ongoing ATG8ylation contributes to the maintenance of Golgi architecture under conditions of chronic microtubule disruption [30]. The strongest evidence for an active repair function came from an acute nocodazole washout assay: after drug-induced Golgi fragmentation, knockdown of genes of LC3 and ATG12 conjugation system, including ATG4B, ATG5, or ATG7, delayed Golgi reassembly, demonstrating that ATG8ylation factors are required for efficient restoration of Golgi architecture [30]. Therefore, Golgi-associated ATG8ylation is an active Golgi repair module rather than a passive readout of organelle stress.

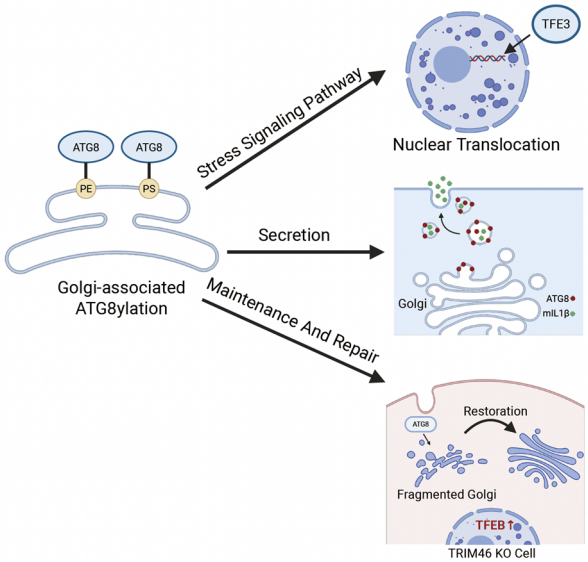

Figure 2. Mechanistic model of the multifaceted functions of Golgi-associated ATG8ylation. Golgi-associated ATG8ylation, marked by ATG8 conjugation to phosphatidylethanolamine (PE) or phosphatidylserine (PS) on

Golgi membranes, mediates three key cellular outputs (PS and PE are depicted as illustrative lipid acceptors in this figure; this lipid-conjugation mode remains experimentally unproven). First, it triggers stress signaling pathways that promote nuclear translocation of the transcription factor TFE3, activating adaptive transcriptional programs. Second, it facilitates unconventional secretion, enabling the release of mature IL1β (mIL1β) via TGN-associated ATG8ylation independent of canonical secretory routes. Third, it supports Golgi maintenance and repair: ATG8 accumulates on fragmented Golgi architecture, accompanied by upregulation of nuclear TFEB, driving the structural restoration of fragmented Golgi under stress conditions. Collectively, these pathways highlight the conserved roles of Golgi-associated ATG8ylation in coordinating stress responses, secretion, and organelle homeostasis. Figure was created in BioRender.com.

A related insight also comes from plants, where heat stress induces a noncanonical role of ATG8 in Golgi recovery [54]. This response requires the core ATG8 conjugation machinery, including ATG5, ATG7, and ATG16, but is independent of upstream autophagy regulators such as ATG1, ATG11 and ATG9 [54]. Although a mammalian-like V-ATPase-ATG16 recruitment mechanism has not been established at the plant Golgi [54], the convergence is striking: in both mammalian and plant systems, stress-responsive ATG8-family conjugation appears to support the preservation of Golgi form and function rather than classical degradative autophagy [54]. Together, these studies suggest that Golgi-associated ATG8ylation may represent a conserved adaptive principle for maintaining organelle architecture under stress.

6. Relationship to Other Golgi Quality-Control Pathways

Golgi-associated ATG8ylation should be integrated into the wider landscape of Golgi quality control, but not conflated with it. Canonical Golgiphagy, which means selective degradation of Golgi components by canonical autophagy, mediated by CALCOCO1 or by YIPF3/YIPF4 receptors, removes Golgi material through autophagosome-dependent degradation during selective remodeling or nutrient stress [42–44]. In addition to CALCOCO1 and YIPF3/YIPF4, TM9SF3 was recently identified as a Golgi-resident ATG8-binding receptor required for Golgiphagy [45]. Notably, TM9SF3-dependent Golgiphagy is activated not only by nutrient stress but also by Golgi stressors including monensin, brefeldin A and perturbation of intra-Golgi glycosylation [45]. Some of these insults also induce Golgi-associated ATG8ylation, raising the possibility that nondegradative Golgi CASM and degradative Golgiphagy may represent distinct, sequential or partially overlapping responses to Golgi dysfunction. In Golgi membrane-associated degradation (GOMED) pathway, Golgi-derived double membranes can generate under selected stress settings from autophagy-deficient cells [55]. Besides, GARD and EGAD represent proteasome-linked Golgi quality control pathways [56,57]. These routes can occur in the same organelle system, but they differ from Golgi-associated CASM in upstream triggers, membrane topology, degradative fate, and likely timescale.

Although this model remains partly inferential, the available evidence is accommodated by a hierarchical framework in which Golgi-associated ATG8ylation acts as an early, rapid, single-membrane remodeling response to Golgi stress or damage. This response may promote TFE3/TFEB-dependent adaptive signaling, facilitate the unconventional secretion of selected cargoes, and support the physical repair of Golgi architecture. If the stress is unresolved or prolonged, this early nondegradative response may subsequently intersect with more terminal degradative pathways, such as canonical Golgiphagy or GOMED.

STING provides an additional link between Golgi trafficking, membrane ion homeostasis, and VAIL. Following activation, STING exits the ER, traffics through the Golgi, and subsequently enters post-Golgi vesicles, where LC3 lipidation requires V-ATPase-dependent recruitment of ATG16L1 through its WD40 domain [38]. STING also possesses a conserved proton-channel activity that promotes deacidification of these vesicles and facilitates VAIL [58,59]. This pathway therefore shares mechanistic features with Golgi stress-induced ATG8ylation and may also converge at the level of downstream effects. In particular, STING-dependent ATG8ylation has been linked to TFEB/TFE3 activation and lysosomal homeostasis, paralleling the transcriptional stress responses associated with Golgi ATG8ylation [60,61]. At present, whether STING contributes to the Golgi-CASM responses induced by DLK1 overexpression, AMDE-1, Golgi fragmentation, or other non-STING stimuli remains unknown. These pathways should therefore be considered mechanistically related but currently distinct manifestations of stress-responsive membrane ATG8ylation.

7. Open Questions and Future Directions

Several key questions now define the next phase of this field. One central issue is how membrane specificity is achieved. Golgi-associated ATG8ylation is often enriched at the TGN, yet the determinants that distinguish

TGN membranes from the cis-Golgi, ERGIC, endosomes, or lysosomes remain unclear. pH gradients, cargo load, ARF/COPI trafficking, and actin-dependent V0-V1 coupling are all plausible contributors, but none alone explains the diversity of reported stimuli. Acute and inducible systems that perturb these parameters individually will therefore be essential for defining how specific Golgi membrane domains become competent for ATG8 conjugation.

Closely related to membrane specificity is the unresolved question of lipid specificity. CASM can conjugate ATG8 proteins to phosphatidylserine as well as phosphatidylethanolamine [15], but whether Golgi-associated ATG8ylation uses the same lipid spectrum as endolysosomal CASM remains unknown. Lipidomic analysis of isolated Golgi/TGN membranes, ideally combined with engineered ATG8 conjugation reporters, could clarify whether phosphatidylserine conjugation is a general CASM signature or whether lipid usage varies according to organelle identity, membrane subdomain, or stress context.

Another important question is how different functional outputs are generated. The same upstream V-ATPase-ATG16L1 machinery has been associated with Golgi stress adaptation, IL1β secretion, and Golgi reassembly [28–30]. These outputs may not be mutually exclusive and could occur in parallel during Golgi stress. One possibility is that an initially similar ATG8ylation event is interpreted by different downstream readers, thereby directing the response toward transcriptional adaptation, secretion, and repair. Alternatively, distinct sub-Golgi membranes, lipid acceptors, ATG8 paralogues, or local signaling environments may generate different molecular codes that bias the pathway toward specific outputs.

A further challenge is to separate Golgi-associated ATG8ylation experimentally from canonical autophagy and Golgiphagy. This distinction is more than technical, because bulk loss of ATG genes affects canonical autophagy, CASM, Golgiphagy, mitochondrial quality control, lysosomal function, and secretion. Future work will require separation-of-function tools that disrupt V-ATPase-ATG16L1-dependent Golgi CASM while leaving canonical autophagy largely intact. Candidate approaches include ATG16L1 WD40-specific mutants, organelle-restricted V-ATPase perturbations, inducible Golgi-targeted ATG16L1 recruitment, and lipid acceptor-selective ATG8 mutants.

The physiological relevance of this pathway is also an important frontier. Golgi stress occurs in infection, inflammation, neurodegeneration, metabolic stress, and secretory cell overload. Golgi-associated ATG8ylation, by linking changes in Golgi membrane state to IL1β secretion, TFE3/TFEB activation, and organelle repair, could therefore be highly relevant to innate immunity and inflammatory disease. Conversely, inappropriate or chronic Golgi-associated ATG8ylation might alter cytokine release, pathogen restriction, protein trafficking, or neuronal Golgi integrity. These possibilities make Golgi-associated ATG8ylation important not only for basic membrane biology, but also for disease-focused research. Although most mechanistic studies of Golgi-associated ATG8ylation have been performed in cultured cells, the oleate model provides important *in vivo* support. Systemic oleate administration induces LC3 lipidation in multiple mouse tissues, and this response remains detectable in Becn1$^{+/-}$ heterozygous mice, demonstrating that oleate-induced LC3 lipidation can occur independently of BECN1 *in vivo* [26]. Cultured-cell studies further show that oleate-induced LC3 accumulation is associated with the TGN and accompanied by blockade of post-Golgi trafficking; consistent with these findings, oleate also inhibits conventional protein secretion *in vivo* [27]. Nevertheless, direct visualization of Golgi-localized LC3 lipidation in tissues, precise identification of the relevant ATG8ylated membrane compartment, and *in vivo* validation of key mechanistic requirements for Golgi-associated CASM, such as V-ATPase and ATG16L1, still require direct validation. *In vivo* studies of ATG16L1-dependent CASM in other settings already point to the disease relevance of this machinery and may provide clues to the potential pathological roles of Golgi-associated CASM. The Crohn's disease-associated ATG16L1 T300A variant is located just upstream of the WD40 domain, increases the susceptibility of ATG16L1 to caspase cleavage, and impairs WD40-dependent interactions and unconventional autophagic functions [62,63]. Thus, T300A provides an important genetic connection between disease susceptibility and functions mediated by the C-terminal region of ATG16L1. More direct evidence for the physiological importance of the WD40 domain comes from experimentally engineered mouse models. Loss of the WD40 domain abolishes LAP-like noncanonical autophagy in pancreatic acinar cells and exacerbates acute pancreatitis [64], whereas disruption of the WD40 domain alters cholesterol and phosphatidylserine trafficking, compromises plasma membrane homeostasis, and increases susceptibility to influenza A virus infection [65]. These findings indicate that WD40-dependent noncanonical functions of ATG16L1 contribute to inflammation, membrane homeostasis, host defense, and tissue protection *in vivo*. However, these phenotypes have not yet been directly linked to Golgi-associated CASM, and naturally occurring disease-associated mutations within the WD40 repeats that selectively affect Golgi ATG8ylation have not been established. In addition, genetic models of Golgi V-ATPase dysfunction may also provide useful systems for testing the physiological relevance of Golgi-associated CASM. Atp6v0a2$^{-/-}$ and proton-transport-defective Atp6v0a2RQ/RQ (R755Q knock-in) mice display elevated trans-Golgi pH, delayed secretory trafficking, glycosylation defects, and impaired Golgi-dependent processes such

as acrosome formation [66]. Although CASM was not examined in these models, they offer a valuable *in vivo* setting in which to determine whether chronic disruption of Golgi acidification and homeostasis alters ATG16L1 recruitment and Golgi-associated LC3 lipidation. Accordingly, future studies combining tissue-specific models, endogenous ATG8 reporters, Golgi-restricted perturbations, and pathway-selective ATG16L1 or V-ATPase mutants will therefore be essential to define whether Golgi-associated ATG8ylation operates *in vivo* and how it contributes to disease-relevant processes.

Together, these studies support a working model in which Golgi membrane stress recruits the V-ATPase-ATG16L1 machinery to license local ATG8 lipidation on single Golgi membranes. Rather than feeding primarily into degradative autophagy, this Golgi-associated ATG8ylation generates nondegradative outputs that help cells adapt to Golgi dysfunction, including TFE3/TFEB-mediated stress signaling, unconventional IL1β secretion, and restoration of Golgi architecture. The field's next steps should include direct lipidomic definition of Golgi-conjugated ATG8 species, higher-resolution mapping of the relevant membrane subdomains, mechanistic dissection of cargo selection during unconventional secretion, and determination of how the pathway interfaces with membrane repair machineries and degradative Golgi quality-control systems. If these questions are answered, Golgi-associated ATG8ylation is likely to become a powerful model for understanding how membrane ATG8ylation functions outside autophagosome biogenesis.

Author Contributions

J.L., Z.X. and M.L.: conceptualization, writing—original draft preparation; M.L.: writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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During the preparation of this work, the authors used AI-assisted tools for language editing and polishing of specific sentences. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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