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Perspective

Feedback at the Core: Protein ATG8ylation as a Regulatory Brake on the Autophagy Engine

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Abstract: Protein ATG8ylation is a post-translational modification (PTM) wherein ubiquitin-like ATG8 proteins covalently conjugate to cellular proteins. In our recent work, we identified ATG7 as a principal substrate of this modification, with K140 as the major site, and demonstrated that ATG8ylation of ATG7 serves as an endogenous regulatory brake on autophagy by disrupting its interaction with ATG3. In this perspective article, we briefly overview the evolution of the protein ATG8ylation field—from its initial discovery to the mechanistic dissection of its core machinery—with emphasis on our recent functional characterization of ATG7 as both enzyme and substrate. We then discuss key unanswered questions, including the search for putative E3 ligases, the stress-responsive landscape of protein ATG8ylation, and emerging links to human disease.

Keywords: protein ATG8ylation; PTM; ATG7; endogenous regulatory

1. Introduction

Macroautophagy (hereafter autophagy) is an evolutionarily conserved intracellular degradation pathway that delivers cytoplasmic constituents to lysosomes for recycling, thereby maintaining cellular homeostasis [1,2]. A central event in autophagosome formation is the covalent conjugation of ubiquitin-like ATG8 proteins (LC3/GABARAP family) to phosphatidylethanolamine/phosphatidylserine (PE/PS) on growing phagophore membranes—a process termed membrane ATG8ylation (lipidation) [3–5]. This modification is catalyzed by a canonical ubiquitin-like cascade involving the E1-like ATG7, the E2-like ATG3, and the E3-like ATG12–ATG5-ATG16L1 complex, and is delipidated by ATG4 family proteases [6–9].

For decades, phospholipids were thought to be the sole physiological conjugation partner for ATG8. However, this view was challenged in 2019 by Agrotis et al. [10], who demonstrated that LC3/GABARAP proteins can also form stable, covalent conjugates with other cellular proteins—a phenomenon now termed protein ATG8ylation. They first characterized the phenomenon and identified ATG4 as the essential deATG8ylation enzyme, with the ATG3 as a primary substrate. Subsequent studies expanded the substrate repertoire: Nguyen et al. [11] reported ATG16L1 as a target during mitophagy, while Jia et al. [12] identified the RNA-binding protein NUFIP2 upon lysosomal damage. These findings collectively suggested that protein ATG8ylation may represent a broader cellular stress response [13]. A major mechanistic advance came from Ketteler et al. [14], who demonstrated that protein ATG8ylation operates independently of ATG5, relying instead on the proteins ATG7 and ATG3.

Despite these advances, fundamental questions remained: What is the physiological function of the novel PTM of protein ATG8ylation? Does this modification actively regulate cellular processes, or merely reflect "aberrant" targeting? Our recent study [15] set out to address these questions, identifying ATG7 as both a principal substrate and a regulatory node, and demonstrating that ATG8ylation of ATG7 serves as an endogenous brake on autophagy. In this Perspective, we bring together these recent discoveries, compare protein ATG8ylation with its membrane counterpart, and discuss the key challenges ahead.

2. Mechanism Solidified: A Distinct Enzymatic Cascade

In our current study [15], we sought to independently verify and significantly expand upon these observations. Using systematic genetic knockout screenings, we confirmed that protein ATG8ylation is indeed independent of early autophagy initiation factors (ULK1, and BECN1 complexes) and the canonical E3 complex, but strictly requires the catalytic activity of ATG7 and ATG3. These findings provide a robust consensus on the core machinery of this PTM. With the core machinery of protein ATG8ylation now clear, we can directly compare this modification with membrane ATG8ylation. This comparison reveals fundamental differences in substrates, enzymatic requirements, and functional outcomes, as summarized in Table 1.

Table 1. Comparison of protein versus membrane ATG8ylation.

	Membrane ATG8ylation	Protein ATG8ylation
Substrate	Phospholipid: PE/PS	Proteins: ATG3, ATG7, ATG16L1, NUFIP2
E1 enzyme	ATG7	ATG7
E2 enzyme	ATG3	ATG3
E3 requirement	ATG12–ATG5-ATG16L1 complex	Independent of ATG5/ATG16L1; unknown E3(s) likely required
Reversibility	ATG4 (delipidation)	ATG4 (deATG8ylation)
Known functions	phagophore expansion, autophagosome maturation, cargo recruitment	autophagy regulation, stress response

Furthermore, our research advances the field by addressing the functional "why". By developing a specialized tandem affinity purification-mass spectrometry (TAP-MS) workflow designed to isolate covalent ATG8-conjugates, we identified ATG7, the E1-like activating enzyme for ATG8, as a principal target of the ATG8ylation process it facilitates. We pinpointed K140 as the specific modification site on ATG7. Intriguingly, our data reveal a modification code: while ATG3 undergoes complex, lysine-dependent poly-ATG8ylation chain, ATG7 is subject to a precise mono-ATG8ylation modification.

Next, by establishing an endogenous ATG7 K140R point mutation cell line, we not only provided direct evidence for the physiological occurrence of endogenous protein ATG8ylation via large-scale immunoprecipitation, but also observed an enhancement in autophagic flux. Mechanistically, we demonstrated that the attachment of ATG8 to K140 site on ATG7 creates a steric or conformational hurdle that disrupts its physical interaction with ATG3. This modification effectively serves as an endogenous "brake", preventing the autophagy engine from over-activating. We demonstrate that protein ATG8ylation functions as a direct, physiological brake on autophagy, thereby extending its role beyond a stress-associated phenomenon to a core regulatory mechanism. While this modification clearly attenuates autophagy by disrupting the ATG7-ATG3 interaction, the precise temporal dynamics of when and how this brake is engaged relative to ATG7's catalytic cycle, remain important questions for future investigation.

3. Future Frontiers: Decoding the protein ATG8ylation Language

While the discovery of ATG7's regulatory role marks a milestone, it likely represents just the starting point for understanding the full scope of this pathway. Several focused directions now warrant immediate investigation:

The Search for Non-Canonical E3 Ligases: The dispensability of the ATG12–ATG5-ATG16L1 complex is a defining feature of protein ATG8ylation. However, our inability to fully reconstitute this modification in *E. coli* expressing only ATG7, ATG3, and LC3 strongly suggests the requirement for unidentified mammalian factors. Are there substrate-specific E3 ligases or potential adaptors that provide the necessary specificity for protein targeting? Unmasking these factors through CRISPR-based genetic screens or interactome mapping during peak ATG8ylation states remains a high-priority challenge.

Mapping the stress-responsive landscape of protein ATG8ylation: The TAP-MS method is a new toolbox. Applying it under various stress conditions (oxidative, metabolic, infection) known to modulate autophagy could reveal a dynamic stress-responsive landscape of ATG8ylation. Does mitochondrial damage, previously linked to increased ATG8ylation [13], recruit a unique set of protein targets? Furthermore, probing patient-derived cells from neurodegenerative disorders like amyotrophic lateral sclerosis (ALS) [16] or Parkinson's disease, could reveal if protein ATG8ylation is dysregulated and contributes to pathology.

4. A Grand New Regulatory Dimension in Cell Biology

Viewed through the evolution of this field—from the first detection of protein ATG8ylation in 2019 to the functional characterization of the ATG7-K140 site in our 2025 study—marks the establishment of a significant new regulatory dimension in cell biology. Protein ATG8ylation is emerging not as a biochemical artifact, but as a complete and self-contained signaling pathway that is central to the maintenance of cellular homeostasis.

The health implications of this pathway are substantial. As a built-in brake on autophagy, protein ATG8ylation likely helps prevent excessive cellular self-digestion and maintain balance. Its dysregulation, however, could contribute to disease in opposing ways: in neurodegenerative disorders such as ALS, excessive ATG8ylation might suppress the clearance of toxic protein aggregates, whereas in established cancers—where autophagy often supports tumor survival—inhibiting this brake could impair stress adaptation.

5. Conclusions and Perspective

The discovery of protein ATG8ylation has fundamentally reshaped our view of autophagy regulation. From the first detection of protein-conjugated ATG8 in 2019 to the functional characterization of the ATG7-K140 site in our 2025 study, the field has established protein ATG8ylation as a complete and self-contained signaling pathway central to cellular homeostasis. Our work reveals that ATG7 is both the activator of ATG8 and a substrate for its own modification, with ATG8 attachment serving as an endogenous brake that limits autophagy. Beyond its brake function, protein ATG8ylation may serve alternative or additional roles. For instance, it maybe represent a reversible storage pool for ATG8, allowing rapid mobilization upon demand; it might act as a recruitment scaffold for other autophagy-related proteins; or, akin to ubiquitin, different ATG8ylation patterns could encode distinct functional outcomes.

Looking forward, several challenges remain. We need to find the E3 ligases that confer substrate specificity, and figure out what mono- versus poly-ATG8ylation chains mean for function. Emerging links to neurodegeneration and cancer suggest that dysregulation of this brake may contribute to disease, pointing to potential new therapeutic strategies. As we map the full set of protein targets under different stress conditions, we expect protein ATG8ylation to emerge as a general regulatory mechanism with implications far beyond autophagy itself.

Author Contributions

H.X.: conceptualization, validation, writing—original draft preparation; M.L.: conceptualization, funding acquisition, project administration, supervision, 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.

Use of AI and AI-Assisted Technologies

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.

Reference

1. Choi, A.M.; Ryter, S.W.; Levine, B. Autophagy in human health and disease. *N. Engl. J. Med.* **2013**, *368*, 651–662. https://doi.org/10.1056/NEJMra1205406.
2. Levine, B.; Kroemer, G. Biological Functions of Autophagy Genes: A Disease Perspective. *Cell* **2019**, *176*, 11–42. https://doi.org/10.1016/j.cell.2018.09.048.
3. Klionsky, D.J.; Schulman, B.A. Dynamic regulation of macroautophagy by distinctive ubiquitin-like proteins. *Nat. Struct. Mol. Biol.* **2014**, *21*, 336–345. https://doi.org/10.1038/nsmb.2787.
4. Kumar, S.; Jia, J.; Deretic, V. Atg8ylation as a general membrane stress and remodeling response. *Cell Stress* **2021**, *5*, 128–142. https://doi.org/10.15698/cst2021.09.255.
5. Deretic, V.; Lazarou, M. A guide to membrane Atg8ylation and autophagy with reflections on immunity. *J. Cell Biol.* **2022**, *221*, e202203083. https://doi.org/10.1083/jcb.202203083.
6. Ichimura, Y.; Kirisako, T.; Takao, T.; et al. A ubiquitin-like system mediates protein lipidation. *Nature* **2000**, *408*, 488–492. https://doi.org/10.1038/35044114.
7. Fujita, N.; Itoh, T.; Omori, H.; et al. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol. Biol. Cell* **2008**, *19*, 2092–2100. https://doi.org/10.1091/mbc.e07-12-1257.
8. Taherbhoy, A.M.; Tait, S.W.; Kaiser, S.E.; et al. Atg8 transfer from Atg7 to Atg3: A distinctive E1-E2 architecture and mechanism in the autophagy pathway. *Mol. Cell* **2011**, *44*, 451–461. https://doi.org/10.1016/j.molcel.2011.08.034.
9. Kauffman, K.J.; Yu, S.; Jin, J.; et al. Delipidation of mammalian Atg8-family proteins by each of the four ATG4 proteases. *Autophagy* **2018**, *14*, 992–1010. https://doi.org/10.1080/15548627.2018.1437341.
10. Agrotis, A.; von Chamier, L.; Oliver, H.; et al. Human ATG4 autophagy proteases counteract attachment of ubiquitin-like LC3/GABARAP proteins to other cellular proteins. *J. Biol. Chem.* **2019**, *294*, 12610–12621. https://doi.org/10.1074/jbc.AC119.009977.
11. Nguyen, T.N.; Padman, B.S.; Zellner, S.; et al. ATG4 family proteins drive phagophore growth independently of the LC3/GABARAP lipidation system. *Mol. Cell* **2021**, *81*, 2013–2030.e9. https://doi.org/10.1016/j.molcel.2021.03.001.
12. Jia, J.; Wang, F.; Bhujabal, Z.; et al. Stress granules and mTOR are regulated by membrane Atg8ylation during lysosomal damage. *J. Cell Biol.* **2022**, *221*, e202207091. https://doi.org/10.1083/jcb.202207091.
13. Carosi, J.M.; Nguyen, T.N.; Lazarou, M.; et al. ATG8ylation of proteins: A way to cope with cell stress? *J. Cell Biol.* **2021**, *220*, e202108120. https://doi.org/10.1083/jcb.202108120.
14. Ketteler, R.; Kiso, K.; von Chamier, L.; Agrotis, A. ATG5 is dispensable for ATG8ylation of cellular proteins. *Autophagy Rep.* **2024**, *3*, 2392450. https://doi.org/10.1080/27694127.2024.2392450.
15. Xie, H.; Long, J.; Huang, Y.; et al. Identification of ATG7 as a protein ATG8ylation substrate. *Cell Rep.* **2025**, *44*, 116485. https://doi.org/10.1016/j.celrep.2025.116485.
16. Torres, P.; Rico-Rios, S.; Ceron-Codorniu, M.; et al. TDP-43 regulates LC3ylation in neural tissue through ATG4B cryptic splicing inhibition. *Acta Neuropathol.* **2024**, *148*, 45. https://doi.org/10.1007/s00401-024-02780-4.