REVIEW ARTICLE

Unraveling the ultrastructure and dynamics of autophagic vesicles: Insights from advanced imaging techniques

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Abstract

Autophagy, an intracellular self-degradation process, is governed by a complex interplay of signaling pathways and interactions between proteins and organelles. Its fundamental purpose is to efficiently clear and recycle cellular components that are damaged or redundant. Central to this process are autophagic vesicles, specialized structures that encapsulate targeted cellular elements, playing a pivotal role in autophagy. Despite growing interest in the molecular components of autophagic machinery and their regulatory mechanisms, capturing the detailed ultrastructural dynamics of autophagosome formation continues to present significant challenges. However, recent advancements in microscopy, particularly in electron microscopy, have begun to illuminate the dynamic regulatory processes underpinning autophagy. This review endeavors to provide an exhaustive overview of contemporary research on the ultrastructure of autophagic processes. By synthesizing observations from diverse technological methodologies, this review seeks to deepen our understanding of the genesis of autophagic vesicles, their membrane origins, and the dynamic alterations that transpire during the autophagy process. The aim is to bridge gaps in current knowledge and foster a more comprehensive comprehension of this crucial cellular mechanism.

KEYWORDS
autophagy, ultrastructure, volume electron microscope

1 | INTRODUCTION

Autophagy, a critical intracellular degradation pathway, is indispensable for cellular homeostasis, facilitating waste elimination and maintaining cellular functionality. This process, vital for cell function, differentiation, and development eliminates and recycles damaged or redundant intracellular proteins, organelles, and components, thereby modulating numerous physiological and pathological events.[1–3] Autophagy is classified into three types: macroautophagy, microautophagy, and chaperon-mediated autophagy (CMA), based on cargo delivery mechanisms. Macroautophagy (hereafter autophagy), a universally conserved process in eukaryotic cells,[4] is extensively characterized in terms of morphology, function, and molecular mechanism. The process typically encompasses several stages: initiation, elongation, closure, maturation, and degradation.[5]

Central to this process are autophagic vesicles (including autophagosomes, amphisomes, and autolysosomes), responsible for isolating, transporting, and degrading intracellular substances.[6,7] In normal conditions, autophagosomes display a unique double-layer membrane structure, encapsulating damaged or dysfunctional organelles and proteins.[8] Upon maturation, these vesicles fuse with endosomes or lysosomes, forming amphisomes or autolysosomes, where degradation occurs.[9,10] The degradation products, such as amino acids and fatty acids, are reused for maintaining cellular health under stress and ensuring cell quality control.[11–13]

Despite decades of research since the initial discovery of autophagic structures in the 1950s,[14] the ultrastructural composition and transformation of autophagic vesicles during autophagosome biogenesis remain elusive.[15] High-resolution nanoscale imaging technologies are essential for observing and analyzing these structures.[16] A comprehensive understanding of autophagic vesicle formation and maturation necessitates integrating complex biochemical and cell biology studies,[16] considering the dynamic nature of their formation and the influence of different cellular environments.[6,8,14]

This review aims to provide an overview of the current knowledge on the ultrastructure of autophagic vesicles, comparing observational characteristics from diverse technological approaches to enhance understanding of autophagy mechanisms and transformations. Additionally, it endeavors to guide future research and offer insights into autophagy's alterations in different physiological and pathological conditions.

2 | THE CONSTRUCTION AND STRUCTURE OF AUTOPHAGIC VESICLES

Autophagic vesicles, including autophagosomes, amphisomes, and autolysosomes are polymorphic and diverse, containing recognizable cytoplasmic components.[6] Their variations are linked to the autophagy's occurrence and progression under different physiological and pathological conditions regulated by numerous autophagy-related genes.[17,18] In response to cellular stress or nutritional deficiencies, autophagic vesicles continuously form, mature, and fuse with lysosomes to address intracellular demands.[8,19] The initiation of autophagy is marked by the emergence of a crescent-shaped, single-layer membrane structure (phagophore) within the cytoplasm. This membrane expands and engulfs cellular materials, eventually forming a nearly spherical, bilayer autophagosome.[20] These substances are isolated within the double-membrane structure, preventing contact with other intracellular components and directing them for degradation. Subsequently, the mature autophagosomes then fuse with lysosomes or late endosomes to form autolysosomes/amphisomes,[5,6,8] where their contents undergo acidic hydrolysis and are recycled into the cytoplasm[10] (Figure 1).

The unique ultrastructure of autophagic vesicles is rational. Autophagosomes dismantle and eliminate intracellular waste and damaged organelles. Their bilayer membranes facilitate formation, fusion, translocation, and ultimate degradation. The inner membrane encircles substrates for degradation, establishing an acidic
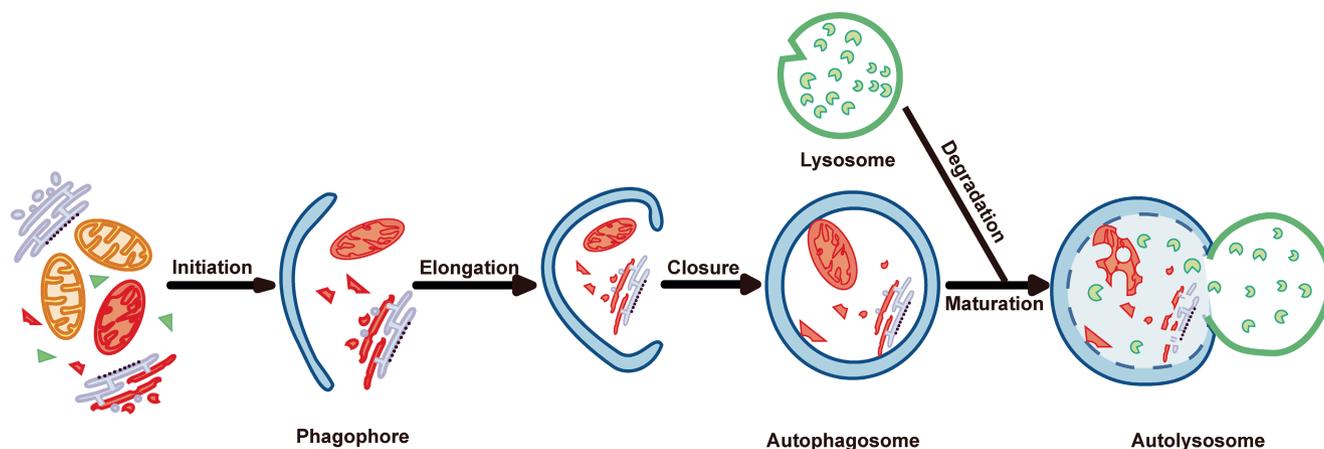

FIGURE 1 Schematic diagram of autophagy in mammals. The process typically includes initiation, elongation, closure, maturation, and degradation. The initiation of autophagy is characterized by the emergence of a crescent-shaped, single-layer membrane structure (phagophore) within the cytoplasm. This membrane expands and engulfs cellular materials such as damaged or dysfunctional organelles and proteins, eventually forming an autophagosome with a nearly spherical bilayer membrane structure. The mature autophagosomes fuse with lysosomes to form autolysosomes, where acidic hydrolysis occurs, leading to the recycling of their contents into the cytoplasm.

microenvironment crucial for lysosomal enzymes. The outer membrane maintains structural integrity and aids in intracellular navigation and fusion with other cellular structures.[21,22] Assisted by cytoskeletal elements, such as microtubules, the outer membrane enables efficient transport of autophagosomes from their genesis to their fusion site, enhancing their efficient union with lysosomes or late endosomes. This sequence facilitates the transfer of materials from the autophagosome to the lysosomes for breakdown and recycling.[23,24] This bilayer structure ensures the efficient execution of the autophagy process, crucial for cellular homeostasis and adaptive to various stresses.

Moreover, another remarkable ultrastructural aspect of autophagic vesicles is their capacity to encapsulate diverse cellular constituents designated for degradation, including defective proteins, organelle fragments, and surplus metabolites.[25] Historically, autophagy was deemed a non-selective degradation pathway, especially under nutrient-deficient conditions. Recent advancements, however, have introduced the notion of "selective autophagy", entailing targeted degradation processes like mitophagy, lysophagy, and ER-phagy.[26,27] In this context, receptor proteins such as p62 (also known as SQSTM1), TAX1BP1, NDP52 (also known as CALCOCO2), etc., target specific cargos, instigating autophagy and orchestrating membrane recruitment, thereby accommodating a variety of substances for degradation.[28] Consequently, autophagic vesicles demonstrate a wide range of shapes, sizes, and functionalities epitomizing the selectivity, adaptability, and versatility inherent in autophagy.

The intricate ultrastructural attributes of autophagic vesicles are pivotal in preserving cellular homeostasis, eliminating damaged cell components, and providing resources during stress. The complexity and dynamics of their morphology stem from multilayered and interconnected regulatory mechanisms within the autophagy process, involving numerous signaling pathways and protein interactions. These features are indispensable for advancing autophagy research and understanding the mechanisms underlying the autophagy process, thereby positioning it as a critical field in cell biology and biomedical studies.

3 | TECHNOLOGICAL ADVANCES FOR THE UNDERSTANDING OF AUTOPHAGIC ULTRASTRUCTURE

3.1 | Optical imaging

The field of optical imaging has significantly expanded our capabilities to visualize the microscopic realm, profoundly impacting the study of autophagy.[29] This advancement encompasses the use of small molecule fluorophores, color fluorescent proteins, and other bioactive compounds often in synergy with various fluorescent probes.[30] Techniques such as fluorescence imaging, bioluminescence imaging, chemiluminescence imaging, and Raman imaging have become instrumental. They facilitate non-invasive, two-dimensional, or multidimensional image data acquisition at both micro and macro scales,[31] enabling detailed characterization of the structural components involved in the dynamic autophagy processes.[32] Particularly, fluorescence bioimaging, a subset of optical bioimaging, has gained substantial traction in the research community. Following Ohsumi's group's initial investigation of autophagy-related genes in yeast, researchers have focused extensively on genes and proteins associated with autophagy, especially the Atg family.[33,34] Fluorescent labeling is pivotal in this context, allowing researchers to anchor targeting objects and trace the dynamic interactions between autophagic vesicles and diverse genes, molecules, or organelles, thereby unraveling the regulatory mechanism underlying autophagy.

The dynamic process of autophagy is illuminated by leveraging fluorescence signals from specific autophagy markers. The microtubule-associated protein 1A/1B-light chain 3 (LC3) exists in two forms—LC3-I and LC3-II—and is integral to the membrane structure of autophagic vesicles.[35,36] Tracking LC3's transformation and accumulation during autophagy under the light microscope reveals multiple bright spot fluorescent signals, aiding in evaluating autophagy activity.[23,37–39] The GFP fluorescent protein's sensitivity to acid, when combined with the mRFP-GFP-LC3 dual-fluorescent plasmid detection method (Figure 2), provides valuable insights into autophagy dynamics based on fluorescence signal color and intensity.[40,41] However, the most of real-time imaging studies investigating autophagic processes have been conducted using in vitro systems. Recent advancements have been made with the development of transgenic mice models, which incorporate a fluorescent protein, such as GFP or Keima, fused to LC3 within their genome and facilitate the visualization of autophagy dynamics via time-lapse microscopy.[42,43] Through the application of these transgenic models, researchers are now capable of dynamically monitoring and tracking the subcellular localization of LC3 in real-time and in situ. Consequently, this enables a more delicate understanding of the dynamic changes occurring during autophagy.

However, fluorescence detection methods for LC3 have limitations, particularly under conditions where LC3 may incorporate protein aggregates, posing challenges in differentiating these aggregates from authentic autophagosomes. Moreover, fluorescence imaging generally limits observations to spot fluorescence or signal overlap and
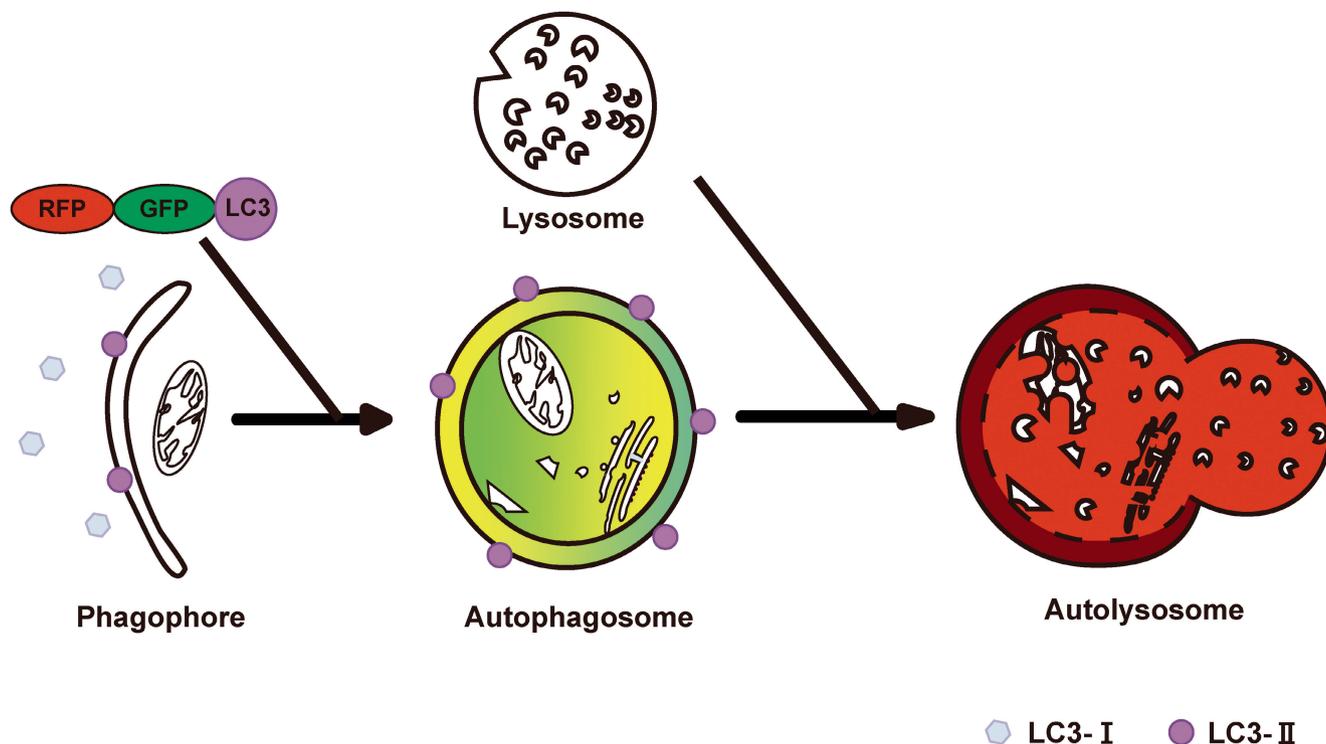
FIGURE 2 Detecting different stages of autophagic vesicles with dual-fluorescent plasmid (mRFP-GFP-LC3). A tandem fusion of mRFP and GFP is employed to tag LC3-II for monitoring the autophagy process. LC3-I predominantly localizes in the cytoplasm. Upon initiation of autophagy, it undergoes phosphorylation to form LC3-II which translocates to the membrane of autophagosomes. During autophagosome formation, multiple bright green or yellow fluorescent spots can be observed. Due to the acid sensitivity of GFP fluorescence protein, upon fusion with lysosomes, the acidic environment attenuates the green fluorescence. Following autolysosome formation, green fluorescence diminishes completely while red fluorescence remains detectable.

contact, obscuring the detailed structure of membrane-bound organelles like autophagic vesicles and cytoskeletal microfilaments. Here, the nanoscale resolution advantage offered by electron microscopy becomes essential for their precise identification, often necessitating a combination of light microscopy (LM) for specific labeling followed by electron microscopy for high-resolution imaging.[44–47]
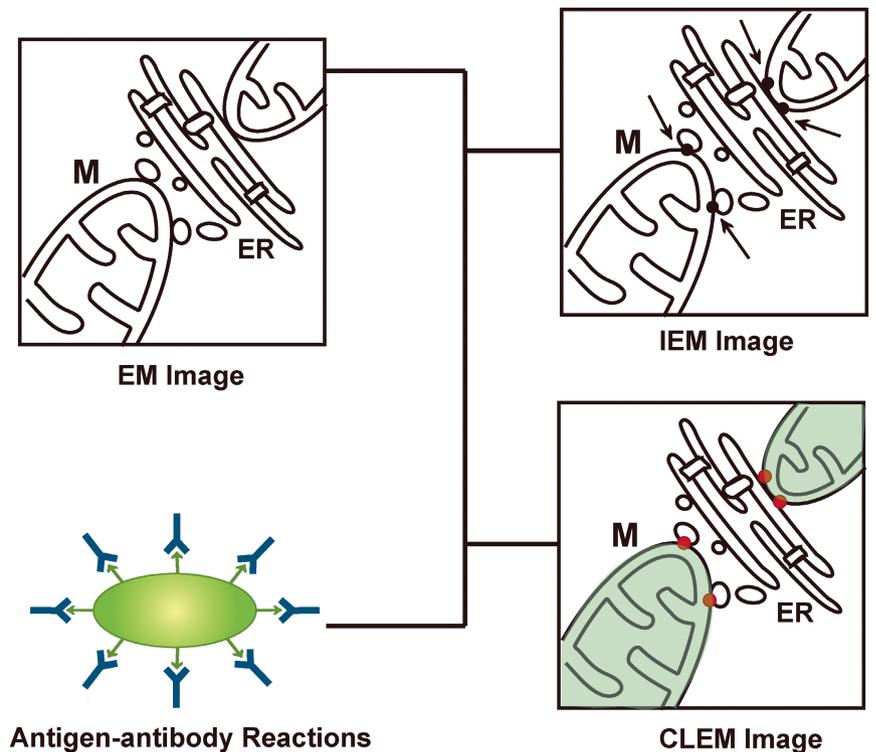
3.2 | Electron microscopy

Electron microscopy (EM), renowned for its exceptional resolution, magnification, and contrast, has been a classic and indispensable technique in exploring cellular and tissue ultrastructure[48–50] (Figure 3). EM's extensive use in autophagic research dates back to the 1950s[51], with the visualization of typical autophagosomes, autolysosomes, or other autophagy-related structures by EM remaining a reliable standard for autophagy measurement.[16]

Pioneering studies, such as Sam Clark's documentation of dense ring-shaped structures housing mitochondria in neonatal mice's proximal tubular epithelium[52] and Alex Novikoff's observation of vacuoles in rat proximal tubules,[53] laid the groundwork for understanding

autophagy. Novikoff further scrutinized the characteristics of the Golgi apparatus and mitochondria surrounding this structure, coinventing the term "cytolysome" to elucidate the nature of this vacuole.[54] Researchers like L. Schneider, Leonard Napolitano, Harald Moe, and Olva Behnke contributed significantly by identifying vacuolar structures containing cytoplasmic elements across multiple organisms. L.Schneider meticulously described "degradation vacuoles" in irradiated paramecia, which potentially degrade the encapsulated cytoplasm.[55] Leonard Napolitano observed vacuoles sequestering entire or partial mitochondria in brown adipose cells of rats exposed to cold and starvation stress.[56] Harald Moe and Olva Behnke captured cytoplasmic bodies containing rough endoplasmic reticulum (ER), mitochondria, and free ribosomes in the intestinal epithelium of newborn rats.[57] Moreover, based on the observation of these vacuoles primarily occurring in pre-lysosome or post-lysosome, Leonard Napolitano proposed that these structures would gradually break down.[56] Thomas and Keith conducted an investigation on rat liver to assess the impact of glucagon and inferred that during the initial formation of vacuoles, the cytoplasmic components surrounding the limiting membrane are surrounded and partially isolated from

FIGURE 3 Comparison of autophagic ultrastructure was observed through electron microscopy (EM), immunoelectron microscopy (IEM), and correlative light and electron microscopy (CLEM). EM's exceptional resolution, magnification, and contrast are sufficient for visualizing the ultrastructure of intracellular organelle membranes. IEM combines antigen–antibody reactions with EM, enabling nanoscale observation of specific proteins. For example, Hamasaki et al. observed ATG14 and ATG5 localization at ER and mitochondrial contact sites (indicated by arrows). CLEM enhances the fluorescence imaging capabilities of light microscopy (LM), allowing for simultaneous fluorescence signal observation and acquisition of high-resolution images.
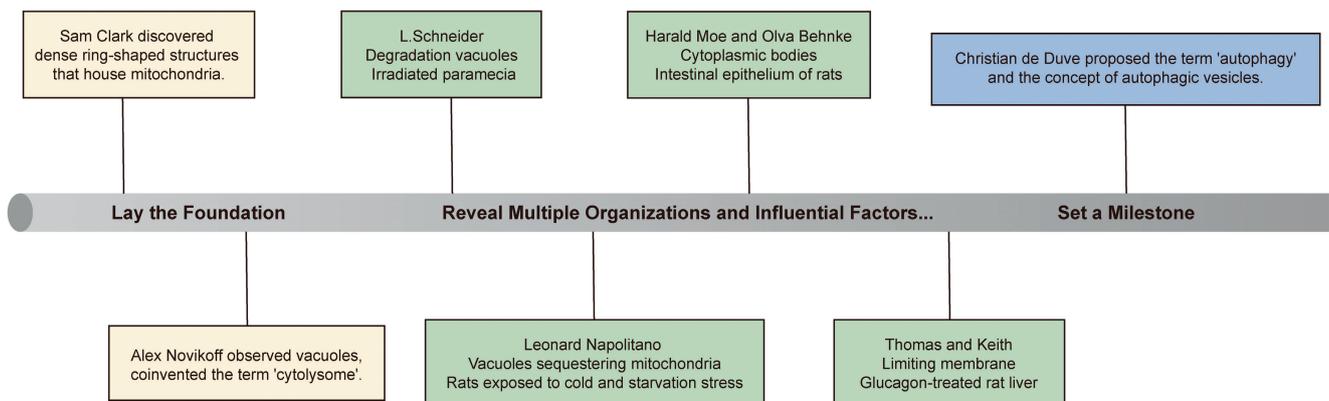

FIGURE 4 The early events of autophagy investigated by electron microscopy. Initially, vesicles were observed in rat renal tissue through EM. Subsequently, numerous researchers have captured similar structures under various tissues and influencing factors. The 1963 CIBA Foundation Symposium on Lysosomes marked a milestone event, formally introducing the term "autophagy" and providing a defined concept for autophagic vesicles.

other intracellular substances.[58] In 1963, the process of focal degradation of intracellular components was elucidated, involving the formation of vacuoles after the initial separation of intracellular components, and ultimately evolving into a lysosome-like body.[59] The 1963 CIBA Foundation Symposium on Lysosomes was a milestone where Christian de Duve proposed the term "autophagy" and established the concept of autophagic vesicles[60] (Figure 4).

Since then, EM has been critical in assessing the dynamic progression of autophagy, enabling direct visualization of morphological alterations in autophagic vesicles at various stages. The non-degrading organelle, the autophagosome, with its bilayer membrane structure, was formally recognized and studied extensively.[61,62] By the turn of the 19th century, EM observations of the autophagic vesicles in diverse physiological and pathological conditions highlighted the functionality and significance

of autophagy.[63] It's crucial to note that improper fixation in EM studies can lead to issues like discontinuity of the autophagic vesicle membrane and difficulty in identifying specific structures, impeding observation and comparison. Thus, proper methodology in EM is vital for accurate and insightful studies in autophagy research.

3.3 | Immunoelectron microscope (IEM)

IEM is a hybrid technique that integrates antigen–antibody reactions with electron microscopy, enabling nanoscale visualization of specific proteins within autophagic vesicles.[22,64] This method allows for precise determination of the distribution and exact localization of target proteins inside autophagic vesicles (Figure 3). Consequently, it provides valuable insights into the origins of the isolation membrane and the dynamic nature of the autophagy process.[35,65]

The research into the origins of the autophagosome, prior to its formal recognition as an organelle, primarily focused on elucidating the mechanisms of cytoplasmic component incorporation and the derivation of the isolation membrane.[61,66] Initially, de Duve et al. hypothesized that the autophagosome originates from pre-existing organelles.[14] Subsequent electron microscopy studies suggested the potential roles of neighboring organelles, such as the ER and Golgi complex, in autophagic vesicle formation.[61,67] Since the late 1980s, IEM has shed light on the origins of these vesicles. Findings have been mixed: some studies indicate an absence of ER-labeled proteins in autophagosome membranes,[68] while others show possible modifications by antibodies against ER proteins.[22] Hamasaki et al. observed ATG14 and ATG5 localization at ER and mitochondrial contact sites in starved HeLa cells (Figure 3), suggesting a complex origin for the autophagosome membrane.[45] These disparate results from electron microscopy and IEM studies have led to a lack of consensus on the membrane's origin, hinting at the possibility of multiple sources or sequential involvement of different organelles in its formation.[69] Another theory posits that autophagosome formation is an emergent process, beginning with a nascent core membrane and expanding through vesicular additions.[70]

IEM also facilitates the classification of autophagic vesicle subtypes. For instance, the sequential degradation of SOD and CAIII in rat liver sections provided insights into the formation and maturation of these vesicles.[71,72] However, the identification of these vesicles under the microscope heavily relies on expert interpretation, underscoring the potential for frequent misidentification errors.[73]

3.4 | Correlative light and electron microscopy (CLEM)

CLEM combines the target-specific imaging capabilities of LM with the high-resolution structural analysis offered by electron microscopy (EM)[74] (Figure 3). Although conceptualized over 50 years ago, its application in autophagy research has only gained momentum recently. CLEM is technically demanding, requiring sophisticated optical systems, meticulous sample preparation, and the integration of various detector types.

Hanson et al. developed an efficient CLEM technique, capturing nearly all GFP-LC3-labeled organelles in a sample.[75] Their approach involved laser scanning confocal microscopy followed by transmission electron microscopy (TEM), conducted on the same dish, significantly enhancing the accuracy of autophagosome identification in cell cultures. CLEM's recent advancements have enabled live cell imaging combined with fluorescent labeling, advancing the study of autophagosome biogenesis and maturation.[76] However, GFP fluorescence decreases in degrading autophagic vesicles, suggesting the use of tandem fluorescence proteins like mRFP-GFP for comprehensive analysis[77] (Figure 2). CLEM has also elucidated the role of specific molecules and organelles in autophagy. For example, Orsi et al.'s investigation into the role of Atg9 protein in autophagy initiation and progression revealed its localization to tubular-vesicular membranes originating from vacuolar structures, highlighting its importance but not as a structural component of autophagosomes.[78] Microautophagy, characterized by autophagic membrane formation within lysosomes, has also been visualized using CLEM, as demonstrated by Omari et al. in their study of misfolded procollagen molecule degradation.[79]

Despite its potential, the broader application of CLEM in life sciences is hindered by complex sample preparation requirements and the need for compatible integration of light and EM technologies. Further, distinct labeling studies are necessary to unravel the complexities of autophagy at various stages.

3.5 | Cryo-electron microscopy (Cryo-EM)

Cryo-EM, an advanced iteration of traditional TEM, incorporates low-temperature transmission and anti-contamination systems. It allows in-situ freezing biological structures, preserving their natural states and enabling atomic-level observation.[80] Cryo-EM encompasses single-particle analysis and Cryo-electron tomography (Cryo-ET) as primary imaging methods.

Cryo-EM has proven instrumental in elucidating cellular architecture,[81] especially in studying the dynamics of autophagy, such as autophagosome assembly and degradation. In-situ Cryo-ET (Table 1), for example, has provided insights into the unique interactions of phagophores with vesicles and the ER during starvation.[15]

Single-particle Cryo-EM has been pivotal in understanding the structure and function of autophagy-associated complexes. Studies like those by Ma et al. on PI3KC3 complexes and Ciuffa et al. on the autophagy receptor p62 have offered profound insights into the molecular architecture and functional dynamics of these complexes.[82,83] Single-particle Cryo-EM provides groundbreaking insights into the initiation, expansion, and substrate-targeting processes underlying autophagosome formation.

However, the advancement of Cryo-EM technology and equipment requires interdisciplinary expertise spanning multiple technical fields. Despite significant progress, further enhancements in resolution continue to pose a challenge, indicating a possible plateau in Cryo-EM development.

3.6 | Optical imaging technology: Surpassing resolution limitations

The direct observation of LC3 expression through fluorescent probes, commonly used for autophagy assessment, is limited by the resolution constraints of traditional optical imaging. This necessitates the utilization of super-resolution fluorescence imaging for characterizing the ultrastructure of autophagic vesicles and associated proteins and organelles.[32] Technologies like confocal microscopy, multiphoton microscopy, and super-resolution microscopy have expanded our understanding of autophagy.

Advanced optical imaging technologies have enabled detailed analysis of autophagy in specific cells. For example, Changou et al. used high-resolution three-dimensional fluorescence imaging to investigate whether autophagosomes must reach a critical size for lysosomal fusion.[84] Similarly, Ligeon et al.'s application of structured illumination microscopy (SIM) provided remarkable insights into protein localization on organelles with 100-nm resolution.[85] While SIM has significantly advanced our understanding of autophagy, its limitations in ultrastructural observation necessitate the concurrent use of techniques like Cryo-EM.[86]

3.7 | Volume electron microscope: observing 3D spatial structures

EM, with its nanoscale resolution, is crucial for observing the ultrastructure of autophagic vesicles. However, traditional EM is confined to two-dimensional projections, which may obscure the comprehensive understanding of autophagic vesicle volumes and dimensions within cells.[51] Volume electron microscopy (vEM), in contrast, allows for creating detailed 3D models by amalgamating images from successive sectioning.[87–89]

TEM, augmented by tomography, has emerged as a pivotal tool in delineating the genesis of autophagic

TABLE 1 Volume electron microscopy reveals autophagic ultrastructure.

Technology	Research objective	Observation result	Implications	Reference
Electron tomography	Relationship of the phagophore membrane and ER	Connections between the phagophore/autophagosome membrane and the closely located ER cisternae	Origin of autophagosome membranes; Mechanism of phagophore membrane extension	88
Electron tomography	Association of isolation membranes (IMs) and ER	ER–IM complex being a subdomain of the ER	Origin and source of autophagosomal membranes	89
Cryo-electron tomography	Structural progression of autophagosome biogenesis	Contact sites between the phagophore and organelles, such as the vacuole and ER	Contribution of different membrane sources; Forces shaping and driving phagophores toward closure	15
Correlative light and electron microscopy with array tomography	Contact between organelles and autophagic ultrastructure	Association of phagophores and ER; ER is the most frequently engulfed organelle	Systematic spatiotemporal analysis of inter-organelle relationships during autophagy	90

vesicles in mammals (Table 1). Pioneering this approach, Yla-Anttila and colleagues undertook EM tomography to scrutinize rat kidney cells under serum and amino acid deprivation. They meticulously captured dual-axis tilt image series from sequential 250 nm sections. Their focus was particularly riveted on a hypothesized linkage between the autophagic vesicle membrane and the ER. Through the reconstruction of 3D tomograms, they elucidated a predominant mode of connection between the phagophores/autophagosomes and adjacent ER cisternae. This connection manifested as a slender extension from the phagophore/autophagosome toward the ER, often with the ER encompassed within the autophagosome.[90] Similarly, Mitsuko et al. utilized EM tomography to examine mammalian cultured cells, uncovering a correlation between early autophagic structures, termed isolation membranes (IMs), and the ER. Their findings suggest that these IMs, emerging as ER subdomains, encase the IMs, forming a structural cradle.[91]

Addressing the challenge of misidentification in EM studies of autophagy, Kit Neikirk and team have introduced a robust method to discern subcellular structures implicated in autophagy (Figure 5). Their technique combines serial block-face scanning EM with Amira software, allowing for a 3D visual representation of the degradation process.[25] Extending this approach, Satoru Takahashi and his team implemented three-dimensional CLEM coupled with array tomography (Table 1). This method was applied to cells subjected to 30-minute starvation, with a focus on the spatiotemporal interplay among organelles during autophagy. Their comprehensive analysis revealed consistent associations of all phagophores with the ER, noting a decrease in ER contact area as phagophores mature into autophagosomes and autolysosomes. Furthermore, they conducted an in-depth examination of the contents within phagophores and autophagosomes, observing frequent targeting of the ER.[92]

4 | CONCLUSION AND PERSPECTIVE

In summary, the integration of classical and advanced imaging methodologies stands as a cornerstone for the detailed exploration of autophagy's dynamic nature and its regulatory mechanisms. This fusion of technologies not only enables a deeper understanding of the intricate processes governing autophagy but also promises to uncover the underlying mechanisms essential for novel therapeutic strategies in diseases where autophagy plays a pivotal role. Advanced imaging techniques such as EM, IEM, and CLEM have significantly expanded our knowledge of autophagic vesicle architecture. Despite these advancements, considerable challenges remain, notably in our comprehension of the selective and heterogeneous nature of autophagy.

Critical areas that require further investigation include cargo recognition and binding mechanisms encapsulated within the "cargo-ligand-receptor" paradigm.[93,94] Elucidating these interactions demands the refinement and application of sophisticated imaging methods that can accurately depict the complex interplay between autophagic components and other cellular structures. Furthermore, the study of microautophagy, hindered by the absence of standardized methods and appropriate models,[95] underscores the necessity for innovative experimental designs, particularly utilizing gene knockout mice to probe the regulatory pathways governing autophagy's various forms. Additionally, although technologies like
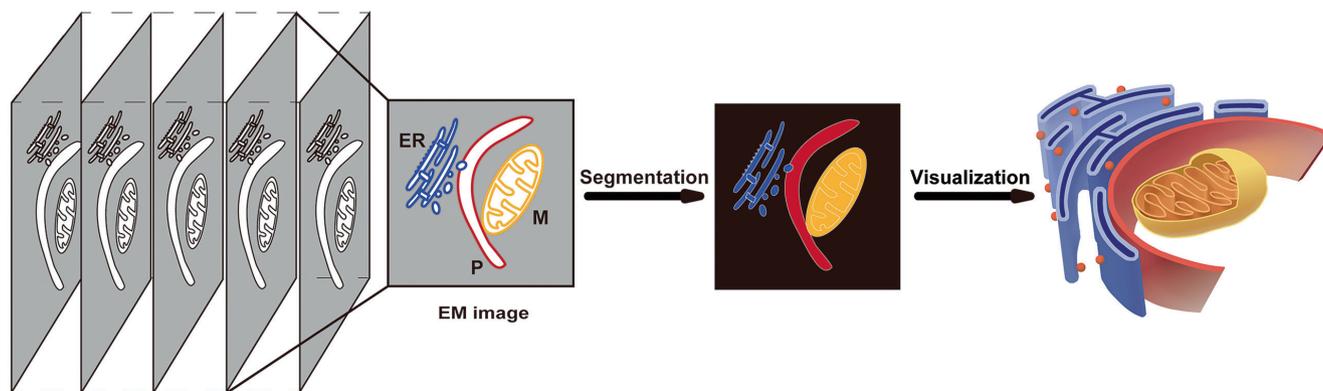

FIGURE 5 Serial block-face scanning electron microscopy (SEM) combined with Amira software for 3D visualization of autophagy process. SEM captures sequential images of target structures by continuous slicing. Then, these images are combined into a comprehensive database in Amira. Subsequently, the contours of the target structure are segmented manually or automatically on a layer-by-layer basis, ultimately resulting in the generation of a precise 3D model. ER, endoplasmic reticulum; M, mitochondria; P, phagophore.

Cryo-ET offer lower resolution compared to Cryo-EM, they provide invaluable insights by preserving the near-physiological state of cells and tissues.[96] This approach, especially when combined with transgenic mouse models, allows for the in situ observation of autophagic structures, thereby enriching our understanding from a unique, physiologically relevant perspective.

In conclusion, it is imperative to leverage these technological advancements not only to dissect the nuanced details of autophagy but also to facilitate the development of therapeutic interventions where modulation of autophagy can yield significant clinical benefits. The continuous evolution of cellular imaging techniques will undoubtedly broaden the horizons for research in cellular biology, offering unprecedented spatial and temporal resolution that could revolutionize our understanding of cellular dynamics and disease pathology.

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