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REVIEW

Physcomitrium patens: an emerging model for autophagy study

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Abstract

Physcomitrium patens (formerly *Physcomitrella patens*), a model moss species, has emerged as an invaluable system for studying autophagy in plants. This review highlights the unique advantages of *P. patens* for autophagy research, including its efficient homologous recombination in mitotic cells, simple body plan, haploid-presiding life cycle, and accessibility to microscopic observation. I discuss recent advances in understanding autophagy mechanisms in *P. patens*, particularly focusing on the role of core autophagy-related (ATG) genes in growth, development, stress responses, and cell death. The characterization of autophagy-deficient mutants revealed unexpected roles of autophagy in promoting cell death under oxidative stress and desiccation, in contrast with classical survival functions. I also examine the conservation and divergence of the autophagy machinery between mosses and vascular plants, emphasizing how *P. patens* bridges evolutionary gaps in our understanding of plant autophagy. Finally, I outline future perspectives on the use of this model system to address fundamental questions about selective autophagy, autophagosome dynamics, and the integration of autophagy with developmental programs.

Keywords Physcomitrium patens · Autophagy · ATG genes · Stress response · Autophagic cell death · Model organism · Bryophyte

Abbreviations

ATG	Autophagy related gene
ACD	Autophagic cell death
ROS	Reactive oxygen species
P. patens	Physcomitrium patens
H_2O_2	Hydrogen peroxide
NH_3	Ammonia
BSA	Bovine serum albumin
LIRs	LC3-interacting regions
AIMs	ATG8-interacting motifs
PCD	Programmed cell death

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Introduction

Autophagy is a highly conserved catabolic process in eukaryotes whereby cellular components are delivered to the vacuole or lysosome for degradation and recycling (Marshall and Vierstra 2018). In plants, autophagy plays critical roles in nutrient remobilization, development, senescence, and responses to biotic and abiotic stresses (Liu and Bassham 2012). The molecular machinery of autophagy consists of approximately 40 autophagy-related (ATG) proteins that orchestrate the formation of double-membrane vesicles called autophagosomes, which engulf cytoplasmic cargo and deliver it to the vacuole for degradation (Li and Vierstra 2012).

While significant progress has been made in understanding autophagy via the model plant *Arabidopsis thaliana*, the moss *Physcomitrium patens* has recently emerged as a complementary and, in some aspects, superior model system for autophagy research. *P. patens* is a bryophyte, representing an early-diverging lineage of land plants that separated from vascular plants approximately 450 million years ago (Rensing et al. 2008). This evolutionary position makes *P. patens*

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particularly valuable for understanding the origin and evolution of autophagy mechanisms in land plants.

The moss *P. patens* offers several unique experimental advantages that make it exceptionally well suited for autophagy studies. First, it exhibits extremely high rates of homologous recombination in mitotic cells, enabling precise gene targeting and the generation of knockout mutants with unprecedented efficiency compared with other plant systems (Schaefer and Zrÿd 1997; Kamisugi et al. 2006). Second, its presiding haploid gametophyte generation allows immediate phenotypic observation of mutations without the need for crossing (Cove 2005; Cove et al. 2009). Third, the simple body plan consists primarily of single-cell-layered tissues that are readily accessible for microscopic observation, facilitating real-time monitoring of autophagy dynamics (Bezanilla et al. 2003). Fourth, protonemal tissue grows by tip growth through continuous cell division, providing an excellent system for studying autophagy in the context of multicellular development and apical growth (Pettinari et al. 2022).

Recent studies utilizing autophagy-deficient mutants in *P. patens* have revealed unexpected and intriguing roles for autophagy in stress responses and cell death, sometimes contrasting with observations in flowering plants (Sakil et al. 2024; Mukae et al. 2023). These discoveries highlight the importance of comparative approaches across different plant lineages to fully understand the complexity and evolution of autophagy functions. In this review, I summarize the current knowledge about autophagy in *P. patens*, emphasizing its unique contributions to the field and discussing how this model system can address outstanding questions in plant autophagy research.

Physcomitrium patens as a model organism

Evolutionary significance and life cycle

Physcomitrium patens, previously known as *Physcomitrella patens*, is a moss belonging to the Funariaceae family within bryophytes (Rensing et al. 2020). Bryophytes represent one of the earliest lineages of land plants, having diverged from the common ancestor of all land plants approximately 450–470 million years ago (Puttick et al. 2018). *Physcomitrium*, which is evolutionarily positioned between vascular plants and green algae, offers important information about how land plants have adapted to environmental stress.

A haploid spore (1n) germinates to start the life cycle of *Physcomitrium*, from which a filamentous structure called protonema (1n) grows. Chloronema and caulonema are the two different cell types that make up protonema (Cove 2005; Rensing et al. 2020). After germination, this early developmental stage is completed in approximately one week. The gametophore (1n), which is made up of rhizoids, stems, and phyllids, then appears and takes on a more intricate structure. The plant achieves this significant developmental milestone in three weeks. The diploid sporophyte (2n) forms at the apex of the adult gametophore during the reproductive phase (Fig. 1). A new life cycle starts when the sporophyte ruptures to release haploid spores after maturation (Harrison et al. 2009; Rensing et al. 2020).

A major experimental benefit of the haploid-presiding life cycle is that mutations in single-copy genes provide instantaneous phenotypic expression without the need for selfing or backcrossing, which are necessary in diploid model plants like *Arabidopsis*. This feature dramatically accelerates genetic studies and is particularly advantageous for studying non-essential genes, including many ATG genes involved in autophagy. For essential genes, where complete knockouts may be lethal, alternative approaches such as RNA interference, conditional knockouts, and inducible expression systems are valuable tools (Bezanilla et al. 2003; Kubo et al. 2019).

Genomic resources and genetic tools

The genome of *P. patens* was first sequenced in 2008, making it the first nonseed plant whose genome has been decoded (Rensing et al. 2008). The latest genome assembly (Bi et al. 2024) contains approximately 480 Mb organized into 26 chromosomes, with over 32,000 predicted protein-coding genes. Comprehensive transcriptomic, proteomic, and metabolomic databases are now available, providing extensive resources for systems biology approaches.

The most remarkable genetic feature of *P. patens* is its extraordinarily high frequency of homologous recombination in mitotic cells, which occurs in 50–90% of transformation events, a phenomenon first quantitatively documented by Schaefer and Zrÿd (1997) in their pioneering analysis of gene targeting efficiency and subsequently confirmed by Kamisugi et al. (2006) through comprehensive studies demonstrating the stability and reproducibility of this exceptional recombination frequency across different nuclear loci. This efficiency far exceeds that of any other plant system and rivals that of yeast, enabling the routine generation of precise gene knockouts, knock-ins, and targeted mutations since the first reverse genetics studies in the late 1990s, when Strepp et al. (1998) demonstrated successful targeted gene replacement in moss protonemata, Girke et al. (1998) established methodologies for stable nuclear transformation and gene targeting, and Schween et al. (2005) further refined these techniques by systematically analyzing factors affecting gene targeting efficiency and developing optimized protocols for generating knockout mutants. This ability has been instrumental in creating autophagy-deficient mutants

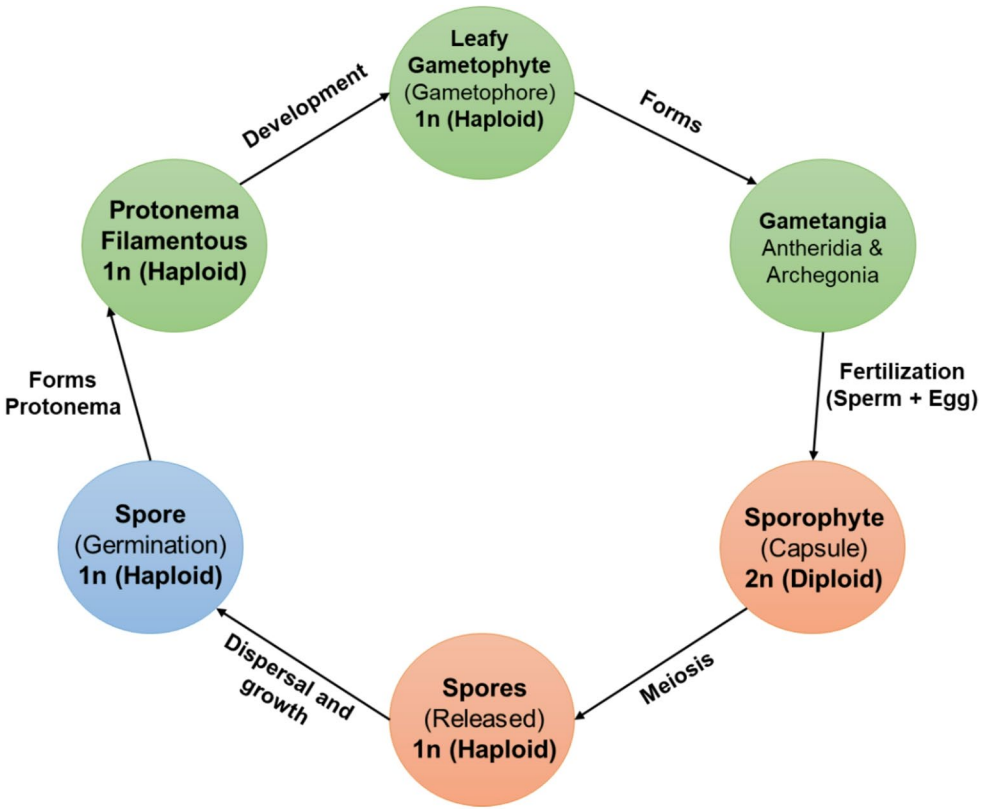


Fig. 1 Life cycle of *Physcomitrium patens*. This diagram illustrates the alternation of generations in *Physcomitrium patens*, a model bryophyte organism. The life cycle alternates between a presiding haploid gametophytic phase (green circles) and a dependent diploid sporophytic phase (orange/coral circles). The leafy gametophore (1n) develops from a protonema filament (1n) and produces gametangia containing antheridia and archegonia. Fertilization of the egg by sperm results in a diploid sporophyte (2n) enclosed in a capsule. Through meiosis, the sporophyte produces haploid spores (1n) that are released and dispersed. The spores germinate to form a new protonema, completing the cycle. Key transitions include gametogenesis (formation of gametes), fertilization, meiosis, spore dispersal and growth, and protonemal development. All labeled structures indicate their ploidy level (1n or 2n) and developmental stage

for functional studies, as exemplified by Mukae et al. (2015) who generated ATG5 knockout lines to investigate autophagy's role in nutrient recycling, Perroud et al. (2018) who developed multiple autophagy-related gene knockouts to study developmental processes, Pettinari et al. (2022) who created targeted mutations in autophagy genes to examine stress responses and cellular degradation pathways, and most recently Mukae et al. (2023) who expanded upon earlier work by generating additional autophagy mutants to elucidate the mechanistic details of autophagy in moss.

Recent advances in genome editing technologies have further enhanced the genetic toolkit available for *P. patens*. CRISPR-Cas9 systems have been successfully implemented and optimized for moss (Mukae et al. 2015, 2023; Lopez-Obando et al. 2016), and more recently, prime editing has been demonstrated in *P. patens*, allowing precise base editing without double-strand breaks (Perroud et al. 2022). These tools complement traditional homologous recombination approaches and enable more sophisticated genetic manipulations, including conditional knockouts and inducible expression systems that are particularly valuable for studying essential autophagy genes.

Cell biology and microscopy advantages

The simple architecture of *P. patens* tissues provides exceptional advantages for cell biological studies of autophagy. The protonemal filaments consist of single-cell files arranged linearly with simple plasmodesmatal connections between adjacent cells, and the gametophore leaves are typically one cell layer thick, making individual cells easily accessible for high-resolution microscopy (Bezanilla et al. 2003). This transparency and simplicity allow real-time observation of subcellular dynamics, including autophagosome formation, trafficking, and fusion with the vacuole, without the need for tissue sectioning or complex sample preparation.

Furthermore, *P. patens* cells are amenable to a wide range of fluorescent markers and reporters. Transgenic lines expressing fluorescent protein-tagged ATG proteins (such as ATG8-GFP) have been successfully generated and used to visualize autophagosome dynamics in living cells (Pettinari et al. 2022; Kanne et al. 2022). The ability to perform long-term live-cell imaging of autophagy processes represents a significant advantage over systems where tissue complexity limits observation.

Protonemal cells also exhibit tip growth, a mode of cell expansion driven by polarized secretion at the cell apex. This growth pattern is shared with other tip-growing cells, such as pollen tubes and root hairs, but differs in that protonemal cells continue to undergo cell division during development (Pettinari et al. 2022). This unique feature allows investigation of how autophagy is spatially and temporally coordinated with cell division, growth, and differentiation in a multicellular context.

Cultivation and experimental manipulation

P. patens is easily cultured under laboratory conditions on simple defined media, either on agar plates or in liquid culture (Cove 2005; Cove et al. 2009; Nishiyama et al. 2000; Mukae et al. 2015). The moss can be propagated vegetatively by mechanical disruption of tissue, with each fragmented cell capable of regenerating a complete plant. This remarkable totipotency facilitates the establishment of clonal populations and the scaling of cultures for biochemical and molecular analyses (Nishiyama et al. 2000).

Environmental conditions such as nutrient availability, light quality and intensity, temperature, and osmotic stress can be precisely controlled, allowing systematic investigations of autophagy under defined conditions. For autophagy research, nitrogen starvation is commonly used to induce bulk autophagy, whereas various stress conditions can be applied to examine selective autophagy and stress-responsive autophagy pathways (Pettinari et al. 2022; Roni et al. 2022).

The transformation of *P. patens* via polyethylene glycol (PEG)-mediated protoplast transformation is straightforward and highly efficient (Schaefer et al. 1991; Mukae et al. 2015). Protoplasts can be generated from juvenile protonemal tissue through enzymatic digestion, transformed, and then allowed to regenerate into complete plants within 4–6 weeks. The high transformation efficiency (typically 10^{-3}–10^{-4} transformants per protoplast) combined with the high rate of homologous recombination in mitotic cells make *P. patens* one of the most tractable plant systems for reverse genetics.

Autophagy machinery in *P. patens*

Conservation of core ATG genes

The core autophagy machinery in plants consists of multiple functional modules that work coordinately to execute autophagosome formation and maturation. These modules include (1) the ATG1/13 kinase complex responsible for autophagy initiation, (2) the phosphatidylinositol 3-kinase (PI3K) complex involved in nucleation, (3) the ATG9-containing vesicle trafficking system, (4) the ATG2-ATG18 complex for membrane expansion, and (5) two ubiquitin-like conjugation systems (ATG8 and ATG12) essential for autophagosome membrane formation (Li and Vierstra 2012).

Genomic analyses have revealed that *P. patens* possesses homologs of most core ATG genes identified in yeast and other plants (Pettinari et al. 2022). The moss genome encodes key components of the autophagy machinery, including ATG1, ATG2, ATG3, ATG4, ATG5, ATG6, ATG7, ATG8, ATG9, ATG10, ATG12, ATG13, and ATG18 family members. The presence of these conserved genes indicates that the fundamental autophagy mechanism was established early in land plant evolution and has been maintained across bryophytes and vascular plants (Table 1).

Phylogenetic analyses suggest that certain ATG gene families have undergone expansion in vascular plants compared with mosses. For example, *Arabidopsis* contains nine ATG8 isoforms, whereas *P. patens* has been reported to contain 3–4 ATG8 genes (Li and Vierstra 2012; Bi et al. 2024), suggesting that the diversification of some autophagy components occurred during the evolution of vascular plants. These differences may reflect adaptations to more complex body plans and developmental programs in flowering plants

Table 1 Core ATG genes characterized in *Physcomitrium patens*

ATG Gene	Function	Phenotype When Disrupted	References
ATG1	Serine/threonine kinase; autophagy initiation	Not fully characterized in moss	-
ATG2	Membrane lipid transfer; autophagosome expansion	Not fully characterized in moss	-
ATG3	E2-like enzyme; ATG8 conjugation	Reduced oxidative stress-induced death	Sakil et al. 2024
ATG4	Cysteine protease; ATG8 processing	Not fully characterized in moss	-
ATG5	E3-like ligase component; ATG8 lipidation	Impaired autophagy; enhanced desiccation tolerance; reduced oxidative stress-induced death	Pettinari et al. 2022; Mukae et al. 2023; Sakil et al. 2024
ATG7	E1-like enzyme; ATG8/ATG12 activation	Impaired autophagy; enhanced desiccation tolerance; reduced oxidative stress-induced death	Pettinari et al. 2022; Mukae et al. 2023; Sakil et al. 2024
ATG8	Ubiquitin-like protein; autophagosome membrane marker	Essential for autophagosome formation	Pettinari et al. 2022; Kanne et al. 2022
ATG9	Transmembrane protein; membrane trafficking	Not fully characterized in moss	-
ATG10	E2-like enzyme; ATG12 conjugation	Not fully characterized in moss	-
ATG12	Ubiquitin-like protein; forms complex with ATG5	Not fully characterized in moss	-
ATG13	Regulatory component of ATG1 complex	Not fully characterized in moss	-
ATG18	PI3P-binding protein; membrane remodeling	Not fully characterized in moss	-

or, alternatively, may indicate specialized autophagy functions that evolved in the vascular plant lineage.

Functional characterization of ATG genes

Reverse genetic approaches have been instrumental in elucidating the functions of ATG genes in *P. patens*. Knockout mutants of core ATG genes, particularly *atg5* and *atg7*, have been generated and extensively characterized (Pettinari et al. 2022; Mukae et al. 2023; Sakil et al. 2024).

ATG5 and ATG7 are components of the ubiquitin-like conjugation systems essential for autophagosome formation. ATG7 functions as an E1-like activating enzyme for both the ATG8 and ATG12 conjugation pathways, whereas ATG5 forms a complex with ATG12 and ATG16, which acts as an E3-like ligase for ATG8 lipidation (Ohsumi 2001). Loss of either ATG5 or ATG7 function completely abolishes canonical macroautophagy, making these mutants ideal tools for investigating autophagy-dependent processes.

Under optimal growth conditions with sufficient nutrients, *P. patens atg5* and *atg7* mutants present relatively subtle phenotypes. The mutants grow and develop normally, producing typical protonemal and gametophore tissues, although some studies have reported slightly slower growth rates or altered protonema morphology (Pettinari et al. 2022). This observation suggests that, unlike in some organisms where autophagy is essential for basal cellular homeostasis, *P. patens* can maintain normal growth and development without autophagy when nutrients are abundant.

However, when subjected to nutrient starvation, particularly nitrogen deprivation, autophagy-deficient mutants present significant defects. Wild-type *P. patens* can survive extended periods of nitrogen starvation by recycling cellular components through autophagy, but *atg5* and *atg7* mutants exhibit accelerated chlorophyll degradation, increased cell death, and impaired recovery when nutrients are resupplied (Pettinari et al. 2022). These findings demonstrate that autophagy in *P. patens* serves a critical survival function under nutrient stress by enabling cellular recycling and maintaining energy homeostasis.

Interestingly, recent studies have also revealed roles for autophagy genes in developmental processes beyond nutrient recycling. Pettinari et al. (2022) demonstrated that autophagy modulates apical growth and tissue differentiation in *P. patens*. The *atg5* and *atg7* mutants presented altered patterns of protonemal development, with differences in chloronema-to-caulonema transitions and changes in cell division patterns. These observations suggest that autophagy is integrated with developmental signaling pathways and may regulate cell fate decisions or provide the local recycling of cellular components necessary for polarized growth.

ATG8 lipidation and autophagosome formation

The ATG8 protein family plays a central role in autophagosome formation. ATG8 proteins are conjugated to phosphatidylethanolamine (PE) in the autophagosome membrane through a ubiquitin-like conjugation system involving ATG7, ATG3, and the ATG5-ATG12-ATG16 complex (Ichimura et al. 2000). Lipidated ATG8 (ATG8-PE) remains associated with both the inner and outer membranes of autophagosomes and serves as a marker for autophagosome identification.

In *P. patens*, ATG8 proteins have been successfully tagged with fluorescent proteins and used to visualize autophagosome dynamics in living cells (Kanne et al. 2022; Pettinari et al. 2022). Confocal microscopy revealed that the number of ATG8-positive punctate structures, which represent autophagosomes and/or autophagic bodies, increased dramatically upon nitrogen starvation or other stress treatments. These structures can be trafficked toward the central vacuole, where they presumably fuse and deliver their contents for degradation.

Interestingly, the overexpression of ATG8 in *P. patens* has been shown to enhance certain cellular processes beyond autophagy. Sanchez-Vera et al. (2017) reported that ATG8 overexpression promotes gamete differentiation, suggesting that increased autophagy may facilitate cellular differentiation and developmental transitions. Kanne et al. (2022) further demonstrated that ATG8 overexpression enhances wound-induced somatic reprogramming, highlighting the remarkable regenerative capacity and cellular reprogramming abilities that characterize *P. patens* biology.

Selective autophagy receptors and cargo recognition

While bulk autophagy involves nonselective sequestration of cytoplasmic contents, selective autophagy targets specific cargoes, such as damaged organelles, protein aggregates, or pathogens, for degradation. This selectivity is mediated by autophagy receptors that recognize specific cargoes and interact with ATG8 proteins through ATG8-interacting motifs (AIMs) or LC3-interacting regions (LIRs) (Marshall and Vierstra 2018).

In vascular plants, several selective autophagy pathways have been characterized, including pexophagy (peroxisome degradation), mitophagy (mitochondria degradation), and chlorophagy (chloroplast degradation). The extent to which these selective autophagy pathways operate in *P. patens* remains an active area of investigation.

Recent studies have begun to identify potential selective autophagy receptors in *P. patens* through bioinformatic searches for proteins containing AIM/LIR motifs and

domains characteristic of cargo receptors (Pettinari et al. 2022; Chen et al. 2020). However, functional validation of these candidates remains limited. Given the transparency of *P. patens* tissues and the availability of organelle-targeted fluorescent markers, this system offers excellent opportunities for live-cell imaging studies of selective organelle autophagy, particularly during developmental transitions or stress responses when organelle remodeling is prominent.

Autophagy in growth, development, and cell division

Autophagy during protonemal development

The protonemal stage of *P. patens* development provides a unique experimental system for investigating autophagy in the context of tip growth coupled with continuous cell division. Pettinari et al. (2022) conducted comprehensive analyses of protonemal growth and development in wild-type and autophagy-deficient mutants under both optimal and nutrient-limited conditions.

Under nutrient-replete conditions, *atg5* and *atg7* mutants presented relatively normal protonemal growth, although some subtle alterations in cell morphology and division patterns were observed. Compared with the wild type, the autophagy-deficient mutants presented slightly lower protonemal extension rates and altered chloronema-to-caulonema ratios. These findings suggest that basal autophagy contributes to optimal growth even under nonstress conditions, possibly by removing damaged proteins or organelles that accumulate during rapid cell division and expansion.

Under nitrogen starvation, the role of autophagy in protonemal development becomes much more pronounced. Wild-type *P. patens* protonemal growth slows but continues for extended periods during nitrogen deprivation, whereas *atg5* and *atg7* mutants exhibit rapid growth arrest and increased cell death (Mukae et al. 2015, 2023; Roni et al. 2022). The chlorophyll content decreased more rapidly in the mutants, and the cellular morphology became abnormal, with increased vacuolation and signs of cellular degeneration. These observations demonstrate that autophagy-mediated nutrient recycling is essential for sustaining protonemal growth when external nutrients are limited.

Interestingly, the spatial distribution of autophagy activity during protonemal development appeared nonuniform. Compared with actively growing tips, fluorescent ATG8 markers result in greater accumulation of autophagosome-like structures in subapical regions, suggesting that autophagy may be compartmentalized along the developmental gradient of protonema (Kanne et al. 2022; Pettinari et al. 2022). This pattern could reflect differential nutrient demands, with older subapical cells engaging in more active recycling to support continued tip growth and cell division.

Autophagy in gametophore development and differentiation

In addition to protonemal development, autophagy also plays important roles during gametophore formation and differentiation. The transition from filamentous protonema to three-dimensional leafy gametophores involves extensive cellular remodeling, including changes in cell shape, division planes, and cellular contents. Sanchez-Vera et al. (2017) reported that autophagy-deficient mutants presented delayed or aberrant gametophore development under certain growth conditions.

Specifically, *atg5* and *atg7* mutants formed gametophores with altered morphology, including changes in leaf shape, size, and cellular organization. The mutants also presented reduced gametophore density and slower developmental progression from protonema to mature gametophore-bearing plants (Sanchez-Vera et al. 2017). These developmental defects are exacerbated under suboptimal conditions, such as reduced nitrogen availability.

The mechanisms by which autophagy influences gametophore development remain to be fully elucidated. One possibility is that autophagy provides recycled building blocks necessary for the rapid cellular proliferation and differentiation that characterize gametophore formation. Alternatively, autophagy might selectively remove specific cellular components whose degradation is required for proper developmental transitions (Sanchez-Vera et al. 2017; Chen et al. 2020). Future studies using cell type-specific markers and high-resolution imaging of autophagy dynamics during gametophore development will help distinguish these possibilities.

Coordination of cell division and autophagy

A particularly intriguing aspect of *P. patens* biology is that protonemal tip cells continue to divide while actively growing, unlike other tip-growing plant cells, such as pollen tubes and root hairs, which typically divide only after growth cessation. This unique feature raises questions about how cellular processes such as autophagy are coordinated with the cell cycle.

Pettinari et al. (2022) reported that autophagy activity may be regulated by the cell cycle in *P. patens* protonemal cells. The quantification of ATG8-positive structures in synchronized cell populations suggested fluctuations in the number of autophagosomes across the cell cycle, with peak autophagy activity occurring during specific phases. However, the detailed relationships between cell cycle

progression and autophagy regulation remain to be fully characterized.

The coordination between autophagy and cell division likely involves multiple regulatory mechanisms. In other systems, autophagy has been shown to be modulated by cell cycle kinases and phosphatases, and autophagy itself can influence cell cycle progression through selective degradation of cell cycle regulators (Sica et al. 2015). Whether similar regulatory circuits operate in *P. patens* is an important question for future investigations.

Autophagy in starch synthesis

Sakil et al. (2022) investigated how autophagy promotes starch synthesis in *Physcomitrium* cells. Researchers have compared wild-type (WT) cells capable of autophagy with autophagy-deficient *atg5* mutant cells to understand the role of autophagy in plant physiology (Sakil et al. 2022).

The study revealed that WT cells accumulated significantly greater starch levels (~12 mg/g fresh weight) than did *atg5* mutant cells when grown on glucose-rich media, despite similar growth rates. This difference resulted from higher starch synthesis rates in WT cells than in *atg5* mutant cells, rather than from differences in starch degradation. When cells are transferred to glucose-deficient medium, both cell types degrade starch at similar rates, suggesting that autophagy specifically promotes starch synthesis rather than preventing starch degradation (Sakil et al. 2022).

This study demonstrated that the starch-promoting effect of autophagy operates through amino acid production. Treatment with an autophagy inhibitor reduced starch synthesis in WT cells, while supplying bovine serum albumin (BSA), which mimics autophagy by delivering proteins to vacuoles for degradation, increased starch synthesis even in *atg5* mutant cells (Fig. 2). Notably, directly supplying glutamine, a free amino acid, did not increase starch synthesis, indicating that cells distinguish between amino acids from autophagy/endocytosis pathways and those from direct nutrient uptake (Sakil et al. 2022).

The mechanism involves rapid amino acid oxidation for energy production. Although total amino acid levels remained unchanged during starch synthesis, the ammonia content increased significantly in WT cells—a byproduct of amino acid oxidation—suggesting that autophagy-derived amino acids are immediately catabolized in mitochondria to generate energy for starch synthesis. Adding BSA to *atg5* cells increased both starch synthesis and ammonia (NH_3) levels, confirming that oxidized amino acids fuel starch accumulation. The study concluded that under nutrient-sufficient conditions, *Physcomitrium* cells prioritize starch storage over growth by using autophagy to produce energy through amino acid oxidation (Sakil et al. 2022).

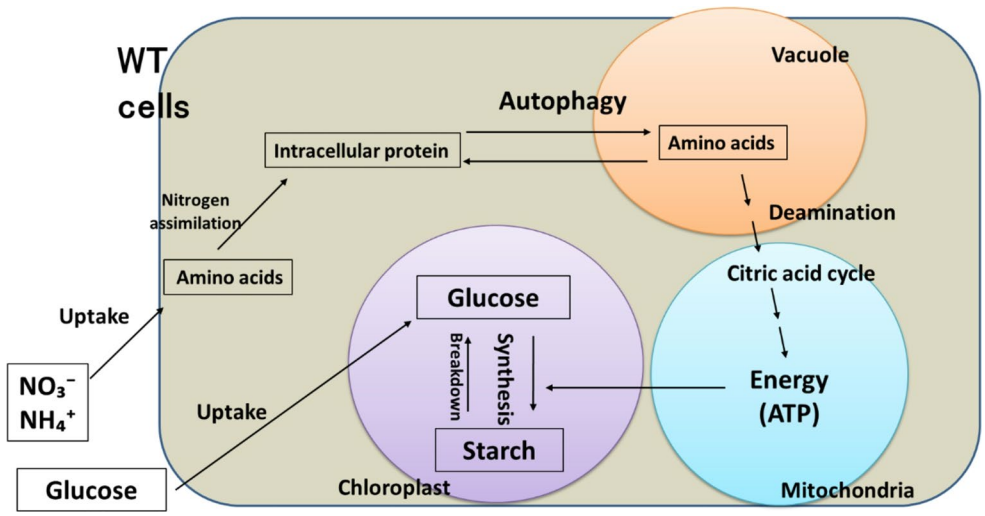


Fig. 2 Role of autophagy in starch synthesis. Schematic representation of the metabolic pathways regulating starch synthesis and energy production in wild-type (WT) *Physcomitrium* cells. The cell illustrates three major interconnected processes: (1) Uptake and assimilation (left): Nitrogen sources (NO_3^- and NH_4^+) and glucose are taken up by the cell and converted into amino acids through nitrogen assimilation. (2) Autophagy and amino acid metabolism (upper right): Intracellular proteins are broken down via autophagy in the vacuole, releasing amino acids. These amino acids undergo deamination, contributing nitrogen skeletons and carbon sources to central metabolic pathways. (3) Carbohydrate metabolism (center): Glucose enters the chloroplast where it is utilized in both anabolic pathways (starch synthesis) and catabolic pathways (breakdown). Starch serves as a major storage carbohydrate. (4) Energy production (lower right): Amino acids derived from protein autophagy are processed through the citric acid cycle in mitochondria, generating ATP to fuel cellular processes. This integrated network demonstrates how autophagy-derived amino acids contribute to both energy metabolism and the supply of carbon skeletons that support starch synthesis in *Physcomitrium* cells

Autophagy in stress responses

Nutrient starvation and autophagy

Nutrient starvation, particularly nitrogen and carbon deprivation, is a canonical inducer of autophagy across eukaryotes. In *P. patens*, nitrogen starvation robustly induces autophagy, as evidenced by increased accumulation of ATG8-positive structures and accelerated delivery of cytoplasmic contents to the vacuole (Pettinari et al. 2022).

Autophagy-deficient mutants show severe sensitivity to prolonged nitrogen starvation. While wild-type *P. patens* can survive weeks of nitrogen deprivation and resume normal growth upon nitrogen resupply, *atg5* and *atg7* mutants exhibit rapid chlorosis, cellular degeneration, and cell death under the same conditions (Mukae et al. 2015, 2023; Roni et al. 2022). Biochemical analyses revealed that mutants fail to maintain adequate amino acid pools and energy reserves during starvation, leading to metabolic collapse (Table 2).

The survival advantage conferred by autophagy during nitrogen starvation presumably reflects its role in recycling nitrogen-containing compounds from dispensable proteins and other cellular components (Hoernstein et al. 2023). This recycled nitrogen can then be reallocated to essential proteins and metabolic processes necessary for survival (Mukae et al. 2015; Roni et al. 2022). Carbon starvation, induced by darkness or treatment with respiratory inhibitors, similarly induces autophagy in *P. patens* and reveals sensitivity in autophagy-deficient mutants (Mukae et al. 2015).

Table 2 Major Autophagy-Inducing conditions in *Physcomitrium patens*

Stress Condition	Autophagy Induction	Phenotype of ATG Mutants	Interpretation
Nitrogen Starvation (Mukae et al. 2015; Roni et al. 2022)	Strong induction	Accelerated senescence; reduced survival	Autophagy provides recycled nitrogen for survival
Carbon Starvation (Darkness) (Mukae et al. 2015; Roni et al. 2022)	Strong induction	Reduced survival	Autophagy maintains energy homeostasis
Desiccation (Mukae et al. 2023)	Strong induction	Enhanced survival (paradoxical)	Autophagy promotes cell death; blocking improves tolerance
Oxidative Stress (H_2O_2) (Sakil et al. 2024)	Strong induction	Reduced cell death (paradoxical)	Autophagy promotes cell death at high ROS levels
Optimal Growth (Pettinari et al. 2022)	Basal levels	Subtle growth defects	Basal autophagy maintains cellular homeostasis

Interestingly, autophagy induction by nutrient starvation appears to involve both transcriptional and posttranslational regulatory mechanisms in *P. patens* (Pettinari et al. 2022). The expression levels of several ATG genes increase upon nitrogen starvation, suggesting the transcriptional upregulation of autophagy capacity. Additionally, posttranslational modifications of ATG proteins, such as phosphorylation, likely regulate autophagy activity in response to nutrient status. The signaling pathways connecting nutrient sensors to autophagy regulation in *P. patens* remain to be fully elucidated but likely involve conserved regulators such as TOR (target of rapamycin) kinase, a master regulator of autophagy in response to nutrient availability.

Desiccation tolerance and autophagy

P. patens is categorized as drought tolerant, salt tolerant, or osmotolerant due to its exceptional resistance to a variety of environmental stressors, including extreme dehydration (up to 92% water loss), high salinity, and osmotic stress (Frank et al. 2005). It can withstand salinity levels that are harmful to the majority of vascular plants. Interestingly, *Physcomitrium* reacts to cold stress via entirely different mechanisms than those in seed plants do, indicating evolutionary differences in stress adaptation tactics (Beike et al. 2015). Abscisic acid (ABA) functions as a central signaling molecule in the stress response mechanisms of *Physcomitrium patens*, demonstrating remarkable evolutionary conservation of ABA-mediated regulatory networks. Research has revealed that ABA-responsive elements (ABREs) and their associated transcriptional regulatory pathways remain functionally conserved in this moss species (Cuming et al. 2007). The ABA signaling cascade in *P. patens* involves complex molecular networks that coordinate stress-induced gene expression, with key components of these pathways showing homology to those found in vascular plants (Arif et al. 2019). Furthermore, studies have identified specific ABA-responsive transcription factors and downstream target genes that are activated during various stress conditions, highlighting the sophisticated nature of hormonal stress signaling even in early-diverging land plants (Stevenson et al. 2016). The conservation of these regulatory mechanisms extends to both the cis-regulatory elements present in promoter regions and the trans-acting factors that bind to them, suggesting that the fundamental architecture of ABA-dependent stress responses was established early in land plant evolution (Lind et al. 2015). This conservation makes *P. patens* an invaluable model system for understanding the ancestral mechanisms of stress hormone signaling and their subsequent elaboration in more complex plant lineages.

Mukae et al. (2023) investigated the role of autophagy in drought and freezing tolerance in *Physcomitrium* mutants.

In contrast to standard autophagy-deficient mutants, which usually exhibit greater sensitivity to environmental stressors, these mutants presented increased resilience to both situations. Research has additionally investigated the function of autophagy in ABA-triggered protective mechanisms. Following ABA treatment, both wild-type (WT) and cells lacking autophagy displayed comparable levels of protective proteins and expression of stress-related genes, suggesting that autophagy does not markedly affect these protective pathways. Both strains fail to endure extreme dryness in the absence of ABA, underscoring the essential function of hormones in stress resistance (Cuming et al. 2007; Koster et al. 2010; Stevenson et al. 2016).

Notably, mutants lacking autophagy retained more water post desiccation, probably because of increased levels of proteins and sucrose, which improved water retention. These mutants presented elevated protein levels prior to desiccation, but the cause of the increased sucrose accumulation after ABA treatment is still unclear. Moreover, autophagy was shown to affect cell death during rehydration. Blocking autophagy decreases cell death rates, implying that autophagy aids in cell death by breaking down cellular components (Fig. 2). Both WT and autophagy-deficient strains underwent cell death during hydration, but WT cells exhibited a higher rate, suggesting that autophagy might worsen damage related to the collapse of the vacuolar membrane (Mukae et al. 2023).

Oxidative stress and autophagic cell death (ACD)

Oxidative stress, caused by the accumulation of reactive oxygen species (ROS), is a common consequence of various environmental stresses, including desiccation, high light, extreme temperatures, and pathogen attack. Autophagy has been implicated in both protecting against oxidative damage (by removing oxidized proteins and damaged organelles) and promoting cell death under severe oxidative stress.

Sakil et al. (2024) investigated the role of autophagy in hydrogen peroxide (H_2O_2)-induced oxidative stress in *P. patens*. Treatment of wild-type mosses with H_2O_2 induced rapid and extensive autophagy, as visualized by increased ATG8 puncta formation and acidification of the vacuole. Surprisingly, autophagy-deficient *atg3*, *atg5* and *atg7* mutants resulted in significantly less cell death than did the wild type under H_2O_2 treatment, demonstrating that autophagy actively promotes cell death under oxidative stress conditions (Sakil et al. 2024).

This prodeath function of autophagy was concentration dependent. At low H_2O_2 concentrations, autophagy provides some protection by removing damaged cellular components. However, at higher concentrations that cause severe oxidative damage, autophagy switches to a pro-death mode, actively contributing to cellular death. This dose-dependent switch suggests that the role of autophagy in oxidative stress responses depends on the severity of damage and may serve as a mechanism for eliminating severely damaged cells that cannot be repaired (Sakil et al. 2024).

The molecular mechanisms underlying autophagy-mediated cell death in *P. patens* likely involve excessive or unregulated autophagy (termed autophagic cell death or autophagy-dependent cell death). Alternatively, autophagy might facilitate programmed cell death (PCD) by degrading survival factors or activating death execution pathways (Fig. 3). Distinguishing these possibilities requires further investigation of the regulatory networks connecting

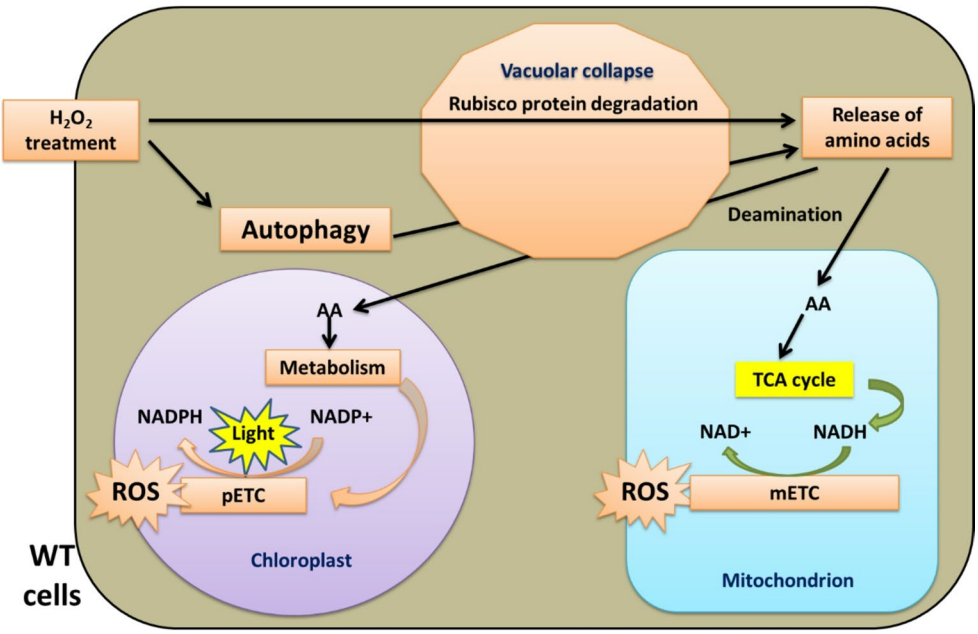


Fig. 3 Stress-induced autophagic cell death. Autophagy is triggered by environmental stressors such as hydrogen peroxide (H_2O_2) treatment, drought, or water stress, which causes amino acids to build up inside vacuoles. These amino acids are then broken down in the mitochondria in tandem with chloroplast activity, which results in increased generation of reactive oxygen species and long-term H_2O_2 buildup. Enzymes for vacuolar processing are also delivered to the vacuole at the same time. High H_2O_2 levels that result cause vacuolar collapse, which in turn leads to programmed cell death

autophagy to the cell death machinery in *P. patens* (Sakil et al. 2024; Sakil et al. 2025).

The findings that autophagy promotes cell death under both desiccation and oxidative stress in *P. patens* represent a paradigm shift from the classical view of autophagy as primarily a survival mechanism (Mukae et al. 2023; Sakil et al. 2024, 2025). These observations highlight the context-dependent nature of autophagy functions and underscore the importance of studying autophagy across diverse stress conditions and organisms.

Comparative analysis: *Physcomitrium patens* versus vascular plants

Conservation and divergence of autophagy components

Comparative genomic analyses between *P. patens* and vascular plants, particularly *Arabidopsis thaliana*, reveal both conservation and divergence in the autophagy machinery. The core ATG genes are generally well conserved, indicating that the fundamental mechanism of autophagosome formation was established early in land plant evolution. However, several ATG gene families have undergone lineage-specific expansion in vascular plants.

For example, *Arabidopsis* possesses nine ATG8 isoforms (ATG8a-i), whereas *P. patens* has been reported to contain 3–4 ATG8 genes (Li and Vierstra 2012; Bi et al. 2024). Similarly, the ATG18 family has expanded in flowering plants, with eight ATG18 members in *Arabidopsis* compared with fewer in moss plants (Li and Vierstra 2012). These expansions may reflect the increased complexity of autophagy regulation in vascular plants or the evolution of specialized autophagy pathways adapted to specific cell types, developmental stages, or stress conditions (Table 3).

Certain autophagy regulators and cargo receptors show more pronounced divergence. For example, the NBR1 (neighbor of BRCA1) protein, an important selective autophagy receptor in *Arabidopsis*, has homologs in *P. patens*, but the degree of functional conservation remains unclear. Similarly, plant-specific regulators of autophagy, such as certain receptor kinases or transcription factors, may differ between mosses and vascular plants.

Functional differences in the roles of autophagy

While the core autophagy mechanism is conserved, functional studies have revealed both similarities and differences in the roles of autophagy between *P. patens* and vascular plants. The protective function of autophagy during nutrient starvation is well conserved across species. In both *P. patens* and *Arabidopsis*, autophagy-deficient mutants exhibit accelerated senescence and reduced survival under nitrogen limitation (Pettinari et al. 2022; Chen et al. 2020; Thompson et al. 2005).

Table 3 Comparison of autophagy characteristics in *Physcomitrium patens* and *Arabidopsis Thaliana*

Feature	Physcomitrium patens	Arabidopsis thaliana
Genetic System	Haploid-presiding; immediate phenotype expression	Diploid; requires homozygosity
Gene Targeting Efficiency	50–90% homologous recombination (in mitotic cells)	< 1% homologous recombination
ATG8 Gene Family Size	3–4 members	9 members (ATG8a-i)
Tissue Transparency	Excellent; single-cell layers	Limited; requires sectioning
Growth Habit	Tip growth with continuous division	Diffuse or tip growth (tissue-specific)
Autophagy Under Nutrient Starvation	Essential for survival	Essential for survival
Autophagy Under Desiccation	Promotes cell death	Generally protective
Autophagy Under Oxidative Stress	Promotes cell death (high doses)	Generally protective
Generation Time	~3 months (spore to spore)	~2 months (seed to seed)
Genome Size	~480 Mb (26 chromosomes)	~135 Mb

However, several notable differences have emerged. The prodeath function of autophagy under desiccation and oxidative stress observed in *P. patens* (Mukae et al. 2023; Sakil et al. 2024) contrasts with findings in some vascular plants, where autophagy typically provides protection against these stresses. For example, autophagy-deficient *Arabidopsis* mutants generally show increased sensitivity to oxidative stress, in contrast to the *P. patens* phenotype (Xiong et al. 2007). These differences suggest that the balance between the prosurvival and prodeath functions of autophagy may differ between bryophytes and vascular plants, possibly reflecting different stress response strategies or distinct regulatory networks controlling the balance between prosurvival and prodeath functions of autophagy.

The developmental roles of autophagy also show both conservation and divergence. In both *P. patens* and *Arabidopsis*, autophagy contributes to senescence and nutrient remobilization during aging (Pettinari et al. 2022; Chen et al. 2020; Yoshimoto et al. 2009). However, the specific developmental contexts differ because of distinct body plans and life histories. *P. patens* offers unique opportunities to study autophagy during tip growth coupled with cell division, a cellular context that is not readily accessible in *Arabidopsis*.

Evolutionary insights from moss autophagy

Studying autophagy in *P. patens* provides valuable evolutionary insights into the origin and diversification of autophagy functions in land plants. The presence of functional autophagy machinery in this early-diverging lineage indicates that while autophagy existed in the algal ancestors of land plants (Laude et al. 2024), it was critical for the colonization of terrestrial environments by the first land plants. Desiccation tolerance, nutrient scavenging in nutrient-poor soils, and oxidative stress management are likely significant challenges during land colonization, and autophagy appears to have been coopted as a key adaptive mechanism.

The differences between moss and vascular plant autophagy may reflect evolutionary innovations that occurred during the diversification of land plants. Some features observed in *Arabidopsis* but absent or less prominent in *P. patens* likely represent derived characteristics that evolved in the vascular plant lineage. Conversely, features present in moss but reduced or modified in flowering plants may represent ancestral characteristics or moss-specific adaptations.

Comparative functional studies between *P. patens*, other bryophytes, lycophytes, and various vascular plants will help reconstruct the evolutionary trajectory of autophagy functions and identify which features are ancestral or derived. Such phylogenetically informed comparisons can reveal how autophagy mechanisms have been modified during plant evolution to accommodate increasingly complex body plans, specialized tissues, and diverse ecological niches.

Molecular regulation of autophagy in *Physcomitrium patens*

Transcriptional regulation

The regulation of autophagy in *P. patens* operates at multiple levels, including the transcriptional control of ATG gene expression. Several studies have documented changes in ATG transcript levels in response to various stimuli, suggesting that transcriptional regulation is an important mechanism for modulating autophagy capacity (Pettinari et al. 2022).

Nitrogen starvation induces the upregulation of multiple ATG genes in *P. patens*, including genes encoding components of the ATG1 kinase complex, the PI3K complex, and ubiquitin-like conjugation systems (Pettinari et al. 2022). This coordinated transcriptional response likely ensures that all components of the autophagy machinery are present at appropriate stoichiometries to support increased autophagic flux during nutrient limitation.

The transcription factors responsible for regulating ATG gene expression in *P. patens* remain largely unidentified. In *Arabidopsis*, several transcription factors, including members of the bZIP, NAC, and WRKY families, have been implicated in autophagy regulation (Michaeli et al. 2014). Whether orthologous transcription factors perform similar functions in moss or whether distinct transcriptional regulatory networks control autophagy represents an important question for future research.

Epigenetic modifications may also contribute to autophagy regulation in *P. patens*. Chromatin remodeling and histone modifications can influence ATG gene expression and cellular responses to stress. The role of epigenetic regulation in moss autophagy remains unexplored but represents an intriguing area for investigation, particularly given the remarkable regenerative capacity and cellular plasticity of *P. patens* (Kanne et al. 2022).

Posttranslational regulation and signaling pathways

In addition to transcriptional control, autophagy is extensively regulated through posttranslational modifications of ATG proteins. The phosphorylation, ubiquitination, acetylation, and lipidation of ATG proteins can modulate their activity, localization, and interactions, resulting in rapid and reversible regulation of autophagic flux.

TOR (target of rapamycin) kinase is a conserved master regulator of autophagy in eukaryotes, acting as a nutrient sensor that inhibits autophagy under nutrient-replete conditions and releases this inhibition during starvation (Dobrenel et al. 2016). *P. patens* possesses TOR kinase homologs, and treatment with TOR inhibitors such as rapamycin or AZD8055 induces autophagy in mosses, suggesting conservation of TOR-mediated autophagy regulation (Saliba et al. 2025). Studies in *Arabidopsis* have demonstrated that TOR acts as a nutrient sensor inhibiting autophagy under nutrient-replete conditions (Caldana et al. 2013).

The sucrose nonfermenting 1-related kinase 1 (SnRK1) pathway provides another major signaling input for the regulation of autophagy in plants. SnRK1 acts as an energy sensor that activates autophagy under energy-limiting conditions. In *Arabidopsis*, SnRK1 can directly phosphorylate ATG proteins and promote autophagy (Soto-Burgos and Bassham 2017). Whether similar mechanisms operate in *P. patens* remains to be determined, but given the conservation of SnRK1 signaling in plants, this seems likely.

Additional signaling pathways involving reactive oxygen species (ROS), calcium, phytohormones (auxin, abscisic acid, ethylene, jasmonic acid), and sugar signals may also regulate autophagy in *P. patens* (Arif et al. 2019; Stevenson et al. 2016). Dissecting these signaling networks and

identifying the key nodes that integrate multiple inputs to control autophagy represent major challenges and opportunities for future research.

Spatial and Temporal control of autophagy

Autophagy is not uniform across all cells or at all times. Instead, autophagy shows spatial and temporal specificity, with different cells or cellular regions exhibiting distinct levels of autophagic activity depending on developmental stage, metabolic state, and environmental conditions.

In *P. patens* protonemata, autophagy activity is greater in subapical regions than in actively growing tips (Pettinari et al. 2022; Kanne et al. 2022). This spatial pattern may reflect differential nutrient demands, with older cells providing recycled nutrients to support continued tip growth. Alternatively, it may indicate the selective removal of damaged components that accumulate during cellular aging, even within the same filament.

The temporal regulation of autophagy is evident during developmental transitions and in response to environmental fluctuations. For example, autophagy increases during the transition from active growth to dormancy or when plants experience sudden nutrient limitation or stress. Cell cycle-dependent fluctuations in autophagy have also been suggested, although a detailed characterization is lacking (Pettinari et al. 2022).

Understanding how autophagy is spatially and temporally controlled requires identification of the regulatory mechanisms that sense local cellular conditions and modulate autophagy accordingly. Live-cell imaging using fluorescent autophagy reporters, combined with optogenetic or chemical-genetic tools to manipulate autophagy in specific cells or at specific times, will be valuable approaches for dissecting this spatial and temporal regulation in *P. patens*.

Technical advantages and methodological considerations

Fluorescent reporters and live-cell imaging

The development of fluorescent reporters for autophagy has revolutionized the field by enabling visualization of autophagy dynamics in living cells. In *P. patens*, several fluorescent protein-tagged ATG proteins have been generated, with ATG8-GFP being the most widely used (Pettinari et al. 2022; Kanne et al. 2022).

ATG8-GFP localizes to autophagosome membranes and appears as punctate structures that increase in number upon autophagy induction. These ATG8-positive puncta can be tracked in real time via confocal or wide-field fluorescence microscopy, providing information about autophagosome formation, movement, and delivery to the vacuole. The transparency of *P. patens* tissues makes it particularly well suited for such live-cell imaging approaches (Kanne et al. 2022).

More sophisticated reporters, such as tandem fluorescent-tagged ATG8 (tfATG8; also called mRFP-GFP-ATG8 or RFP-GFP-ATG8), have been developed for flux measurements. This reporter takes advantage of the differential pH sensitivity of GFP (quenched in acidic environments) and RFP (stable across pH ranges). Autophagosomes appear yellow (GFP+RFP+), whereas autophagic bodies inside the acidic vacuole appear red only (GFP-RFP+), allowing distinction between autophagosomes and completed autophagy events. The implementation of such tandem reporters in *P. patens* would greatly facilitate quantitative analyses of autophagic flux.

Other useful reporters include vacuolar pH indicators (such as pH-sensitive GFP variants targeted to the vacuole) that can monitor vacuolar acidification as a readout of autophagy-mediated cargo delivery and organelle markers (mitochondrial, peroxisomal, or chloroplast-targeted fluorescent proteins) to study selective organelle autophagy.

Biochemical and molecular approaches

In addition to imaging approaches, biochemical methods provide quantitative measurements of autophagy activity. Western blot analysis of ATG8 lipidation (the conversion of ATG8-I to ATG8-II upon lipid conjugation) is a standard assay for monitoring autophagy. In *P. patens*, ATG8 lipidation increases upon nitrogen starvation or other autophagy-inducing conditions and is abolished in *atg7* and *atg5* mutants (Pettinari et al. 2022).

Monitoring the degradation of autophagy substrates provides another measure of autophagic flux. Long-lived proteins, which are preferentially degraded by autophagy, can be labeled metabolically, and their degradation rate can be measured. In autophagy-deficient mutants, the degradation of long-lived proteins is significantly reduced, providing functional evidence of impaired autophagy (Marshall and Vierstra 2018).

Proteomic approaches, including quantitative mass spectrometry, offer powerful tools for identifying autophagy substrates and for characterizing proteome changes in autophagy-deficient mutants. Such unbiased approaches can reveal unexpected roles of autophagy in cellular metabolism and identify new pathways regulated by autophagy. The availability of comprehensive proteomics resources for *P. patens* makes it well suited for such systems-level analyses (Perroud et al. 2018).

Transcriptomic approaches (RNA-seq) can identify genes whose expression changes in autophagy-deficient

mutants or upon autophagy induction, providing insights into autophagy-regulated processes and compensatory responses. The integration of transcriptomic, proteomic, and metabolomic data provides a holistic understanding of the cellular functions of autophagy (Pettinari et al. 2022).

Genetic and chemical tools

The exceptional efficiency of gene targeting in *P. patens* makes it the premier plant system for reverse genetics. The generation of knockout mutants, epitope-tagged lines, and conditional expression systems is routine. CRISPR-Cas9 and prime editing further expand the genetic toolkit, enabling multiplexed gene editing, base editing, and precise modifications (Lopez-Obando et al. 2016; Perroud et al. 2022).

For studying essential genes or for the temporal control of autophagy, inducible systems are valuable. Chemical-genetic approaches using inducible promoters (such as β-estradiol-inducible or ethanol-inducible systems) have been developed for *P. patens* and can be applied to regulate ATG gene expression temporally (Kubo et al. 2019).

Chemical modulators of autophagy provide complementary tools. TOR inhibitors (rapamycin, AZD8055, and Torin1) induce autophagy (Saliba et al. 2025), whereas autophagy inhibitors such as concanamycin A (which blocks vacuolar acidification) and wortmannin (which inhibits PI3K) can block autophagic flux. These compounds enable acute manipulation of autophagy without genetic perturbation, which is useful for dissecting rapid responses and signaling cascades.

Emerging technologies such as proximity labeling (APEX, BioID) for identifying protein interaction networks and optogenetic tools for the spatiotemporal control of signaling pathways hold great promise for future autophagy research in *P. patens*. The genetic tractability and optical clarity of moss make it an ideal system for implementing these cutting-edge approaches.

Conclusions

Physcomitrium patens has emerged as a powerful and versatile model system for plant autophagy research, complementing studies in *Arabidopsis* and other models. The unique experimental advantages of mosses—including efficient gene targeting in mitotic cells, haploid genetics, simple anatomy, and optical transparency—enable investigations that are difficult or impossible in other plant systems. Studies in *P. patens* have revealed unexpected roles for autophagy in promoting cell death under desiccation and oxidative stress, challenging conventional views of autophagy as primarily a prosurvival mechanism. These findings highlight the context-dependent and sometimes paradoxical nature of autophagy functions.

The evolutionary position of *P. patens* as an early-diverging land plant provides unique opportunities to trace the evolution of autophagy mechanisms and understand how autophagy contributes to the colonization of terrestrial environments. Comparative analyses between moss and vascular plants reveal both conserved core machinery and lineage-specific innovations, illuminating the evolutionary trajectory of autophagy in plants.

P. patens is poised to address fundamental questions about selective autophagy, autophagy-cell death interactions, spatiotemporal regulation of autophagy, and the integration of autophagy with broader cellular networks. The continued development of genetic tools, imaging technologies, and systems biology approaches in this model organism promises to yield new insights into the mechanisms and functions of autophagy in plants. As we deepen our understanding of autophagy in *P. patens*, we not only learn about this fascinating moss but also gain general principles broadly applicable to plant biology, with potential implications for crop improvement and sustainable agriculture.

Limitations and future directions

Despite the remarkable advantages of *P. patens* as a model system for autophagy research, several limitations and knowledge gaps remain to be addressed. While the core autophagy machinery has been well characterized, our understanding of selective autophagy pathways—including mitophagy, pexophagy, and chlorophagy—remains limited in moss compared with vascular plants. The identification and functional validation of cargo receptors that mediate selective autophagy represent a critical priority, particularly given the unique evolutionary position of *P. patens*. Additionally, although autophagy-deficient mutants have revealed unexpected prodeath functions under oxidative and desiccation stress, the molecular mechanisms underlying the switch between prosurvival and prodeath remain poorly understood. Future studies should focus on identifying the regulatory nodes that determine autophagy's context-dependent outcomes and characterizing the signaling networks that integrate autophagy with programmed cell death pathways. The application of emerging technologies, such as proximity labeling for mapping ATG protein interaction networks, optogenetic tools for spatiotemporal control of autophagy, and single-cell transcriptomics to capture cellular heterogeneity during stress responses, will be instrumental in addressing these questions. Furthermore, extending comparative analyses to include additional bryophyte species,

lycophytes, and basal vascular plants will provide deeper evolutionary insights into how autophagy mechanisms have been modified across land plant lineages. Finally, translating fundamental discoveries from *P. patens* to crop species could inform strategies for improving stress tolerance and nutrient use efficiency in agriculture, although the extent to which moss-specific autophagy functions are conserved in flowering plants requires systematic investigation.

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Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

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