

REVIEW

The critical role of autophagy in plant responses to abiotic stresses

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Abstract Autophagy is an evolutionary conserved recycling process in eukaryotes whereby intracellular components are engulfed by autophagosomes, which are subsequently transferred to the vacuoles for further degradation and reuse. In organisms like yeast and metazoans, autophagy is actively engaged during environmental perturbation either by degrading denatured proteins and organelles or by interfacing with stress related signaling molecules. Studies over the last decade have also revealed numerous important mechanisms where autophagy is widely involved in plant abiotic stress responses. Autophagy serves as a pivotal route for nutrient remobilization by the degradation of superfluous or damaged cellular cytoplasmic material and organelles. It is also reported to regulate the accumulation of reactive oxygen species, to maintain the cellular redox balance of plants under stressful conditions. Furthermore, autophagy is essential in regulating cellular toxicity by removing aggregated and/or denatured proteins and thereby improving plant stress tolerance. In this review, recent advances in our understanding of autophagy, along with pathways and regulatory networks through which it influences many aspects of plant growth and development in response to nutrient starvation, oxidative stress, osmotic stress and extreme temperatures are discussed.

Keywords abiotic stresses, autophagy, extreme temperature, nutrient starvation, osmotic stress, oxidative stress

1 Introduction

Autophagy is an evolutionary conserved degradation process in eukaryotes that breaks down cellular components for recycling during developmental transitions as well as under stress conditions^[1,2]. In organisms, autophagy can be divided into microautophagy^[3], macroautophagy^[4], chaperone-mediated autophagy^[5], based on the specific process and mechanism, while types of organelle-specific autophagy are classified according to the specificity of substrates^[6]. In plants, microautophagy and macroautophagy have been reported. Microautophagy occurs by invagination of the tonoplast into vacuoles^[3]. Basic research on autophagy in eukaryotes has primarily focused on macroautophagy, hereafter referred to as autophagy. In plants, autophagy is initiated by formation of double-membrane vesicles called autophagosomes which engulf the intracellular contents. Afterwards, the outer membrane of autophagosome fuses with the vacuolar membrane, forming a single-membrane structure known as an autophagic body, which is degraded by hydrolases in the vacuole^[7,8].

Autophagy is executed by autophagy-related (*ATG*) genes. The first *ATG* was discovered in yeast (*Saccharomyces cerevisiae*); subsequently many *ATGs* were identified in different plant species, including *Arabidopsis*, tobacco (*Nicotiana tabacum*), rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), wheat (*Triticum dicoccoides*), maize (*Zea mays*), and apple (*Malus domestica*) based upon their sequence similarity with yeast *ATGs*^[1,9,10]. In plants, more than 30 *ATG* genes have been identified and grouped depending on the intensive functional investigation of their counterparts in yeast, such as the Atg1/13 kinase complex; the vacuolar-protein-sorting (VPS)-34 phosphatidylinositol 3-kinase complex; the Atg9/2/18 transmembrane complex and the entire Atg8/12

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conjugation system^[1,11]. The process of autophagy is regulated by TOR (targets of rapamycin) kinase signaling^[12]. TOR prevents integration of Atg1 and Atg13 via phosphorylation of Atg13. When TOR is inhibited, Atg13 can associate with Atg1, which then promotes phosphorylation and triggers autophagy^[13]. Phosphatidylinositol 3-kinase complexes, including VPS34, VPS15 and Atg6/Beclin1, are essential for the induction of vesicles and nucleation^[14]. Atg9 is localized in the preautophagosomal structure (PAS) and non-PAS structures, and it is thought to deliver lipid to the forming autophagosomes^[1,15]. The movement between PAS and non-PAS requires the participation of Atg2 and Atg18. The Atg8/12 ubiquitin-like conjugation systems mediate the expansion of the autophagosome membrane and cargo engulfment^[15]. The C-terminal of Atg8 protein is first cleaved by Atg4, a cysteine protease, exposing a glycine residue, which then binds to an E1-like enzyme, Atg7^[16]. Latter, the Atg8 is transferred to Atg3, an E2-like enzyme. At last, the Atg8 is conjugated to the membrane lipid phosphatidylethanolamine (PE) to form an Atg8-PE complex, which can be cleaved by Atg4 for further recycling^[1,14]. Similarly, Atg12 first binds with Atg7, is then transferred to another E2-like enzyme, Atg10, and finally conjugates with Atg5, forming an Atg12-Atg5 complex^[17,18]. In yeast, Atg12-Atg5 further interacts with Atg16^[4]. An Atg16-like protein has been reported in *Arabidopsis* and maize^[9,19], but its function remains unclear in plants. Finally, the Atg8-PE conjugates and Atg12-Atg5 complexes promote autophagosomes expansion and movement toward vacuoles^[20].

The regulation of autophagy in yeast and animals has been thoroughly investigated^[4,21], but knowledge of this in a plant system still remains fragmentary. Use of RNA interference (RNAi) to reduce the expression of *AtTOR* results in an upregulated expression of some *ATGs* and constitutive activation of autophagy, which is largely dependent on *AtATG18a* in *Arabidopsis*, indicating that TOR is an antagonistic regulator of autophagy in plants^[22]. NBR1 (neighbor of the *BRCA1* gene), the first isolated selective autophagy receptor with a ubiquitin-association domain in plants, interacts with both Atg8 and ubiquitin, and is involved in the integration of ubiquitinated protein aggregates for degradation through autophagy^[23,24]. NBR1 is essential for plant tolerance to abiotic stresses, such as heat, oxidative, salt and drought stress, but has no function in age- and darkness-induced senescence and resistance to necrotrophic pathogen^[23]. Furthermore, tomato HsfA1a (heat shock transcription factor A1a) has a crucial role in drought tolerance by increasing the transcript levels of *ATG10* and *ATG18f* coupled with induction of autophagosomes formation^[25]. Atg18a interacts with WRKY33 transcription factor to mediate the formation of autophagosomes and resistance to necrotrophic pathogens, while silencing of *WRKY33* compromises the tolerance to *Botrytis cinerea*^[26,27]. In addition, reactive oxygen species (ROS) also mediate the activation

of autophagy in plants^[28]. Treatment with hydrogen peroxide in *Arabidopsis*, promotes the expression of autophagy-related genes and increases the number of autophagosomes^[29]. Autophagy is blocked by NADPH oxidase inhibitors during nutrient and salt stress, but not in osmotic stress^[30]. Although the current understanding of regulation and mechanism of autophagy in plants is poor, it's operation in response to various environmental stresses has been investigated by genetic and molecular analyses of knockout and/or knockdown mutants^[2,11,31]. Using the existing knowledge, this review provides an overview of recent advances in our understanding of the regulation of autophagy in plants as a response to diverse abiotic stresses.

2 Role of autophagy in plants challenged with abiotic stresses

2.1 Nutrient deficiency and induced-senescence

In plants, autophagosomes engulf cellular contents and transfer their cargo to the vacuole for degradation and reuse to meet the needs of cells under nutrient deficient conditions^[32]. Sucrose starvation in suspension-cultured cells is used as a model system to analyze plant autophagy^[8]. When tobacco suspension-cultured BY-2 cells are grown in a sucrose-free medium for 2 d, more than 30% of the intracellular proteins are degraded^[33]. In addition, the perinuclear regions of the cytosol of carbon-starved cells accumulate a number of small spherical bodies after treatment with the cysteine protease inhibitor E-64c for 8 h^[33]. Takatsuka et al. investigated the role of 3-methyladenine, an inhibitor of autophagy in mammalian system, in tobacco culture cells under sucrose starvation and found that 3-methyladenine can inhibit the formation of autophagosomes even in the absence of sucrose^[34]. Carbon starvation arrests cell growth and transiently induces the expression of *AtATG3*, *AtATG4a*, *AtATG4b*, *AtATG7* and *AtATG8* family genes and the formation of autophagosomes in *Arabidopsis* suspension-cultured cells at the onset of starvation^[35]. Furthermore, under sucrose starvation conditions extensive autophagy can be triggered to degrade cytoplasmic constituents in rice suspension cells^[36]. Thus, autophagy is necessary for cell survival during nutrient deficient conditions.

In *Arabidopsis*, *ATG* defective mutants, such as *atg2* and *atg5*, exhibit an early-senescence phenotype even under nutrient-rich conditions^[37] and the *atg* mutants are hypersensitive to nutrient-limiting conditions^[38]. The *atg7* and *atg9* plants display accelerated senescence in nitrogen- and carbon-free medium, characterized by premature chlorosis of the mature rosette leaves and a gradual decrease in chlorophyll content^[18,39,40]. Additionally, plants lacking *ATG13* and *ATG18a* are hypersensitive to nutrient deficiency and senesce, similar to other mutants

missing components of the autophagy system^[41,42]. Phillips et al. found that *atg10-1* mutated plants are more sensitive to nitrogen and carbon starvation than wild-type (WT), as characterized by elevated leaf senescence and programmed cell death (PCD), which are accompanied by failure to accumulate autophagosomes in the vacuole^[18]. The rate of primary root elongation, total number of lateral roots per primary root and the total length of lateral roots per primary root in *Atatg4a4b-1* double mutant plants are remarkably low in the absence of nitrogen in comparison to WT ones, suggesting that autophagy is required for root elongation under nutrient starvation conditions^[16]. In addition, Atg11 interacts with the Atg1/13 complex by promoting vesicle delivery to the vacuole and *atg11* mutant plants exhibit hypersensitivity to nitrogen and fixed-carbon limitations, and compromise the activation of general autophagy and senescence-induced mitophagy^[43]. Therefore, autophagy is involved in regulating nutrient utilization and plant development in the absence of nitrogen or carbon. Other organisms show a similar phenotype to *Arabidopsis* under nutrient starvation conditions. The maize *atg12* mutants show inhibited growth, elevated leaf senescence and compromised autophagic bodies formation during nitrogen or fixed-carbon starvation^[9]. Starvation dramatically induces the expression of *ATG8i* in soybean^[44]. Pérez-Pérez et al. investigated the molecular characterization of autophagy in *Chlamydomonas reinhardtii* and demonstrated that cell aging and nutrient limitation could increase the abundance of Atg8^[45]. On carbon or nitrogen starvation medium, *Physcomitrella atg5* mutants display an early-senescence phenotype compared with WT, which is due to the lack of cytoplasmic degradation by autophagy leading to amino acid imbalance^[46]. These results suggest that autophagy plays a critical role in response to nutrient stress for nitrogen and carbon mobilization.

Autophagy is a conserved process of bulk degradation, and nutrients recycling in all eukaryotes. Autophagy can selectively target plant organelles, such as chloroplasts and endoplasmic reticulum (ER), and their contents broken down by autophagic bodies, which is vital not only in plant development but also in response to various environmental stresses^[47]. Wada et al. identified the role of autophagy disruption in affecting the number and size of chloroplasts during senescence under dark^[48]. The number and size of chloroplasts in individually darkened leaves of WT plants decreased, but this was compromised in *atg4a4b-1* plants^[48]. Furthermore, while chloroplast stroma can be transported into the vacuole by Rubisco-containing bodies (RCBs), a specialized type of autophagic vesicle, this process is not observed in the vacuoles of *atg* mutant plants^[49–51]. The RCBs are detected in rice during energy limitation, while they are inhibited in the presence of an inhibitor of autophagy or in an *ATG7* knockout mutant^[50]. Tunicamycin or dithiothreitol induces ER stress-activated autophagy in *Arabidopsis* root cells, and fragments of ER

can be engulfed by autophagosomes for degradation in vacuoles, a process mediated by inositol-requiring enzyme 1b, one of the ER stress sensors^[47]. Taken together, upon nutrient starvation, the activity of TOR is inhibited which subsequently promotes Atg13 association with Atg1 and Atg11 to trigger autophagy for engulfing cellular components, including mitochondria, RCBs and fragments of ER, which are then transferred to the vacuole for degradation by hydrolases for nutrient reuse (Fig. 1).

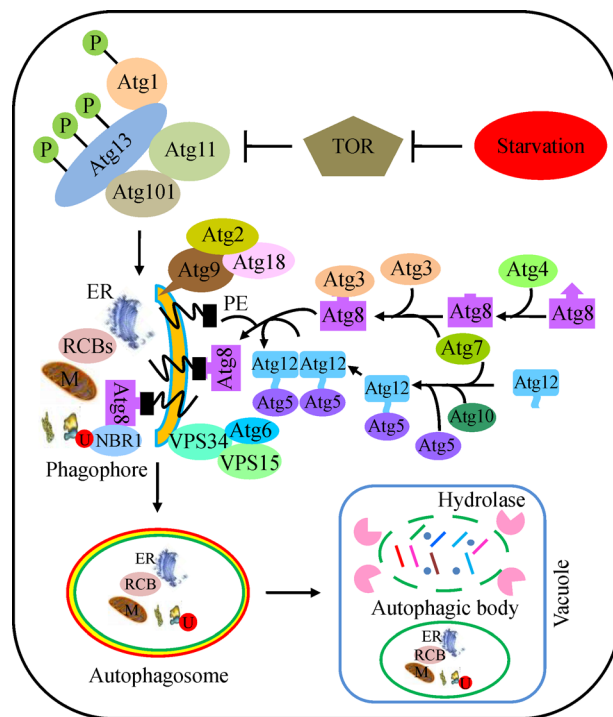


Fig. 1 Schematic representation of induction of autophagy by nutrient starvation in plants. When plants are exposed to deprived nutrient conditions, the activity of TOR is inhibited and subsequently promotes Atg13 association with Atg1 and Atg11 to induce formation of autophagy for engulfing cellular components including mitochondria, RCBs and fragments of ER, and then transfers them to the vacuole for degradation by hydrolases for nutrient reutilization. Atg, autophagy-related gene; P, phosphate group; TOR, targets of rapamycin; ER, endoplasmic reticulum; PE, phosphatidylethanolamine; RCBs, Rubisco-containing bodies; M, mitochondria; U, ubiquitin; NBR1, neighbor of the *BRCA1* gene; VPS, vacuolar-protein-sorting.

2.2 Role in oxidative stress

The production of ROS such as singlet oxygen, superoxide anion and H_2O_2 is increased during environmental stresses^[52]. In plants, ROS accumulation is controlled by the balance between production and scavenging through enzymatic and non-enzymatic mechanisms. The ROS-scavenging enzymatic system contains vital enzymes such as ascorbate peroxidase, catalase, dehydroascorbate reductase, glutathione peroxidase, glutathione peroxidoredoxin, glutathione reductase, monodehydroascorbate reductase,

and superoxide dismutase. The systems of non-enzymatic scavengers include potent antioxidants, such as ascorbate and glutathione^[53]. Under severe stress conditions, the ability to remove ROS through ROS-scavenging systems becomes weakened, resulting in excessive ROS accumulation, which leads to further oxidative stress and can cause damage to cellular components, including proteins, lipids and DNA^[28].

Numerous studies have reported that oxidative stress can induce autophagy both in animals and plants^[28]. Autophagy-defective mutants of yeast accumulate higher levels of H₂O₂ than WT cells during nitrogen starvation conditions, and the excessive ROS can impair the respiratory pathway resulting in cell death^[54]. These studies suggest that autophagy is essential for scavenging ROS and maintaining mitochondrial function under nutrient starvation. Treatment of *Arabidopsis* with H₂O₂ results in oxidation of proteins and induction of autophagy^[29]. However, *AtATG18a*-RNAi plants are hypersensitive to H₂O₂ and accumulate more oxidized proteins than WT plants due to a lower rate of degradation by autophagy, indicating that autophagy is vital in removing degraded proteins under oxidative stress^[29]. Consistent with these results, Yoshimoto et al. also demonstrated that *atg2* and *atg5* mutants accumulated higher levels of H₂O₂ compared to WT plants^[37]. In addition to H₂O₂, treatment of *Arabidopsis* with methyl viologen, a kind of ROS-producing agent that receives electrons from PSI in chloroplasts and then reacts with oxygen to produce superoxide that is rapidly converted to H₂O₂, also activates autophagy^[29]. Thus, ROS can activate autophagy, which in turn is important for the scavenging ROS-induced oxidized proteins. In addition, ER stress promotes the production of ROS and the accumulation of unfolded/misfolded proteins, leading to oxidative stress and induction of autophagy in singlet oxygen resistant1 mutant of *Chlamydomonas reinhardtii*^[55]. Carotenoid-deficient mutants have low photoprotection ability, which results in high levels of ROS in the chloroplast, triggering autophagy^[56]. Recent research has shown that submergence and ethanol treatments enhance the levels of ROS produced by NADPH oxidase in the rosettes of *atg* mutants, which is required for adaptation to submergence and ethanol stress, and activation of autophagy^[57]. Inhibition of NADPH oxidase activity significantly suppressed the induction of autophagy by ROS, suggesting that NADPH oxidase induces production of H₂O₂ and has a partial role in triggering autophagy^[56]. Zhang et al. found that cadmium stress induced the production of ROS that might act as a secondary messenger to promote the formation of autophagosomes^[58]. In close agreement, *atg* mutants are more sensitive and exhibit more serious damages, such as higher oxidative damage and more ROS accumulation than WT plants during aluminum stress^[59]. Silencing of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an important enzyme in energy metabolism, results in

ROS accumulation and activation of autophagy through inhibition of the interaction of Atg3 with GAPDH^[60,61]. Interestingly, melatonin strongly increases the formation of autophagosomes under oxidative stress in *Arabidopsis* seedlings^[62]. Moreover, rice *atg10b* mutants are hypersensitive to methyl viologen and accumulate high levels of oxidized proteins^[63]. *nbr1* mutant plants also show compromised resistance to oxidative stress, like *atg5* and *atg7* autophagy-defective plants^[23]. This implies that autophagy plays a critical role in plant adaptation to oxidative stress by degrading oxidized proteins.

2.3 Role in osmotic stress

Osmotic stress such as salt and drought stress is the most common environmental stress that severely affects plant growth and development^[64]. Globally, more than 6% of arable land and 30% of irrigated areas are subject to salinity-related problems^[64]. With the change of global climate, the area of both salinity- and drought-prone areas will be increased in the future. Although salt stress and drought stress have some common features they are differently regulated in plants. Salt stress acts as both an ionic and osmotic stress for plants^[65], whereas osmotic stress enhances the accumulation of ROS, causing membrane disruption, enzyme dysfunction and oxidation of proteins^[11]. As described above, autophagy plays an important role in scavenging oxidized proteins, indicating that autophagy might be involved in the response to osmotic stress.

Autophagy is identified as mediating enhanced tolerance to salt and drought stress. The expression of *AtATG18a* and the formation of autophagosomes are induced under both salt and drought conditions in *Arabidopsis*^[30]. The growth of WT and RNAi-*ATG18a* seedlings is inhibited after salt and drought treatment, but this inhibition is more severe for RNAi-*ATG18a* plants, which display a chlorosis phenotype with salt application and high level of anthocyanin under drought stress^[30]. In addition, induction of autophagy blocks ROS formation by applying an NADPH oxidase inhibitor during salt and drought stress^[30]. NADPH oxidase inhibition can suppress the activation of autophagy under salt stress, but it has no effect on autophagy under drought stress^[30]. These results indicate that autophagy is regulated by NADPH oxidase-dependent and independent pathways. Recently, it was found that drought can upregulate numerous *ATG* genes and promote autophagosomes formation in tomato^[25]. *HsfA1a*-silenced plants are sensitive, while the over-expressing (OE) plants are more tolerant toward drought stress. Drought stress increases the levels of insoluble proteins in *HsfA1a*-silenced plants, and is associated with downregulation of *ATG10* and *ATG18f* and decreased formation of autophagosomes^[25]. Furthermore, electrophoretic mobility shift assay (EMSA) and chromatin immuno-precipitation coupled with qPCR (ChIP-qPCR)

analysis reveal that HsfA1a can directly bind to the promoters of *ATG10* and *ATG18f*. Silencing of *ATG10* and *ATG18f* in WT and *HsfA1a* OE plants compromises autophagosome formation and *HsfA1a*-induced drought tolerance^[25]. Thus, HsfA1a upregulates *ATGs* expression and autophagosomes formation increases drought tolerance. Similarly, drought stress significantly increases the transcript level of *ATGs* and autophagosome formation in wheat, rice and apple^[10,66,67]. Li et al. analyzed the role of *SiATG8a* in foxtail millet (*Setaria italica*) in response to drought and nitrogen starvation^[68]. Consistent with other research, the expression of *SiATG8a* is dramatically induced under drought stress^[68] and overexpression of *SiATG8a* in *Arabidopsis* increases survival rates, chlorophyll content, and proline content when compared with WT plants^[68]. In summary, salt and drought stress induce the expression of *ATG* genes and promote the formation of autophagosomes to remove denatured proteins and impaired organelles, which results in enhanced stress tolerance.

2.4 Role in extreme temperatures

As with other abiotic stresses, extreme temperatures, such as heat and cold, impair plant growth and development^[69]. Exposure to heat or cold results in the accumulation of insoluble and oxidized proteins^[23,70], which in turn induce autophagy. Yang et al. showed that heat stress activates autophagy resulting from accumulation of unfolded proteins in ER that in turn contribute to ER stress^[71]. In *Arabidopsis*, *atg5*, *atg7* and *nbr1* mutants increase electrolyte leakage, but decrease the values of maximum quantum yield of PSII (Fv/Fm) during heat stress, indicating that the membrane integrity and the capacity of PSII photochemistry of *atg* and *nbr1* mutants are more sensitive than WT plant under heat stress^[23]. Moreover, the *atg* and *nbr1* mutants accumulate enhanced levels of insoluble and detergent-resistant proteins that are highly ubiquitinated in comparison to WT plants under heat stress^[23]. Similarly, heat stress induces the expression of *ATG5*, *ATG7* and *NBR1* in tomato and the formation of autophagosomes^[72]. *ATG5*-, *ATG7*- and *NBR1*-silenced tomato plants show increased symptoms of heat shock, electrolyte leakage and suppress the formation of autophagosomes after heat treatment, suggesting that these knockdown plants are sensitive to heat stress^[72]. In addition, both exogenous as well as endogenous melatonin enhances thermotolerance, which is associated with lowering the levels of insoluble and ubiquitinated proteins due to increased expression of heat-shock proteins, several *ATG* genes and autophagosome formation to re-fold/degrade denatured and unfolded proteins during heat shock^[73]. These results indicate that melatonin promotes protection of cellular proteins to enhance heat tolerance. Cheng et al. investigated the relationship between 2-Cys peroxiredoxins (2-CP) and autophagy during heat stress in

tomato^[74]. Virus-induced gene silencing of tomato genes *2-CP1*, *2-CP2*, and *2-CP1/2* results in compromised heat tolerance, but increases the expression of *ATG5* and *ATG7* and the formation of autophagosomes^[74]. Likewise, *ATG5*- and *ATG7*-silenced plants exhibit high levels of the transcript and increased abundance of 2-CP protein^[74]. Thus, 2-CP interacts with autophagy to enhance tomato heat tolerance. Furthermore, the number of upregulated *CaATG* genes and autophagosomes in a pepper (*Capsicum annuum*) thermo tolerant variety was greater than in a thermo sensitive variety^[75]. Cold stress also increases the transcript level of numerous *ATG* genes and the formation of autophagosomes in pepper^[75]. However, several *ATGs* are inhibited under cold stress in tobacco^[76]. All these studies suggest that autophagy is actively involved in the plant's responses to extreme temperature stress.

3 Conclusions and future perspectives

Over recent decades, the identification of autophagy-related genes and the vital roles of autophagy in the response to different abiotic stresses have been well established^[11,32]. In this review, we summarize the current research on and existing knowledge of the function of autophagy in plants compromised by abiotic stresses, such as nutrient starvation, oxidative stress, osmotic stress and extreme temperatures. A large number of studies demonstrate that transcription factors (TFs), stress responsive hormones, calcium ions and ROS have pivotal roles in plant responses to various abiotic and biotic stresses^[77,78]. As shown in Fig. 2, the production of ROS increases under abiotic stresses and ROS-scavenging weakens, resulting in an impaired cellular redox balance due to excessive ROS accumulation, which further accelerates the accumulation of unfolded and oxidized proteins. Furthermore, abiotic stresses enhance the accumulation of denatured and unfolded proteins (Fig. 2). These damaged proteins trigger activation of autophagy for degradation of the denatured and unfolded proteins, thereby enhancing stress tolerance. In contrast, ROS generated by abiotic stresses may act as molecular signals to induce autophagy (Fig. 2). Stress-induced TFs, such as HsfA1a, can directly bind to the promoters of *ATG* genes to activate autophagy under environmentally perturbed conditions (Fig. 2). In addition, the activity of TOR is inhibited under nutrient starvation and the autophagy pathway is subsequently activated (Fig. 1).

Although the pivotal role of autophagy in the stress tolerance has been thoroughly investigated, the regulation and molecular mechanisms still remain poorly understood. Consequently, several important issues related to plant autophagy still need to be addressed. First, it was found that tomato HsfA1a upregulates the expression of *ATG10* and *ATG18f* and promotes autophagosome formation to increase drought tolerance^[25]. Whether other TFs can also

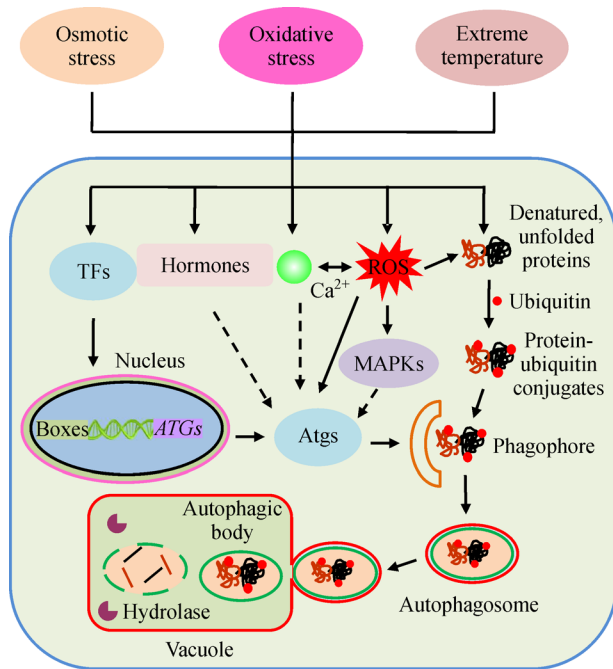


Fig. 2 A proposed model for the role and regulation of plant autophagy in response to osmotic, oxidative and extreme temperature stresses. Abiotic stresses, such as osmotic stress, oxidative stress and extreme temperature, increase the activity of TFs, the content of stress response hormones, the concentration of Ca^{2+} in the cytoplasm, the production of ROS and the accumulation of denatured and unfolded proteins. ROS can act as signaling molecules to activate autophagy, MAPKs and Ca^{2+} , which in turn activates ROS. Excessive ROS further induces accumulation of oxidized proteins that are then ubiquitinated. In addition, TFs bind to the promoters of *ATG* genes and directly regulate their expression to induce the formation of autophagosomes to engulf the ubiquitinated proteins for breakdown by hydrolase in the vacuole. The potential regulation of autophagy by hormones, Ca^{2+} and MAPKs is shown in this model. TFs, transcription factors; *ATGs*, autophagy-related genes; ROS, reactive oxygen species; MAPKs, mitogen-activated protein kinases.

regulate autophagy in responses in plants facing stresses, how these TFs regulate autophagy, and their possible functions in plant development and stress tolerance require further investigation. Second, H_2O_2 induced by NADPH oxidase increases the activation of c-Jun N-terminal kinase, the homologues of plant MAPKs (mitogen-activated protein kinases), which in turn promote the induction of autophagy in animal cells^[79,80]. In plants, NADPH oxidase-dependent production of H_2O_2 can elevate the activity of MAPK1/2^[81,82]. The autophagy-related protein sequences contain a large number of phosphorylation sites. Whether MAPKs interact with autophagy-related proteins and how the MAPKs regulate autophagy need to be addressed (Fig. 2). Third, calcium released from ER and lysosomes activates a Ca^{2+} signaling pathway to induce autophagy in animal cells^[83,84]. The

regulation of autophagy by Ca^{2+} in plants is still a matter of investigation (Fig. 2). Last, autophagy is regulated by hormones in development and disease resistance in animals^[85,86]. Whether hormones are involved in the regulation of autophagy in plants (Fig. 2) and the mechanism also needs to be studied.

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Compliance with ethics guidelines Yu Wang, Jie Zhou, and Jingquan Yu declare that they have no conflicts of interest or financial conflicts to disclose.

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