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CELLULAR AND MOLECULAR MECHANISMS CONTROLLING AUTOPHAGY: A PERSPECTIVE TO IMPROVE PLANT STRESS RESISTANCE AND CROP PRODUCTIVITY

(review)

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Abstract

Under stress conditions, crops cannot reach the maximal level of productivity. Moreover, stress very often leads to plant death. Various stress factors limit the development and success of agricultural praxis. Under stress conditions, plants generate multicomponent metabolic, physiological and genetic responses which help them to adapt to suboptimal environment. At the level of cells, recent research has demonstrated that part of cellular content can be 'eaten' by the cell upon stress, producing energy and metabolites for survival. This process is known as autophagy (J.H. Hurley et al., 2017). Apart from this, some cells can die in the course of so-called programmed cell death (PCD), to provide better conditions for survival of other cells under stress (W.G. van Doorn et al., 2011). Both these processes are highly conservative in the evolution of eukaryotic organisms; they are very important for plant stress response and survival in suboptimal environment. Both autophagy and PCD are being intensively studied in yeast and animals since 1960ies. In plants, studies of autophagy and PCD began rather recently, and it should be kept in mind that these processes in plants bear several important features, which distinguish them from similar processes in heterotrophic eukaryotes. These features are related to the peculiar structures of plant cells. Nowadays, the problem of crop resistance to drought, salinity and extreme temperatures has become especially acute in a number of regions. Therefore, research on stress-induced autophagy is of special interest, as this process is most probably a universal component of the stress response to the abovementioned factors (V. Demidchik et al., 2017; M.E. Pérez-Pérez et al., 2017). Unraveling the mechanisms regulating the stress-induced autophagy and PCD may provide a key to genetic and chemical control of plants stress resistance, life cycle and productivity. Constitutive (i.e. not induced by stress) autophagy is an important mechanism of renewal of defect cell components; in plants, enhancement of autophagic flux by overexpression of the genes encoding autophagy-related proteins leads to an increase in stress resistance and to delayed senescence. In course of plant development, many types of plant cells undergo autophagy followed by PCD at the terminal stage of differentiation. In particular, autophagy and PCD are indispensable for seed germination, formation of vascular system and development of generative organs. Autophagy also participates in the regulation of leaf and petal senescence. So-called 'nocturnal' autophagy takes part in the degradation of transient leaf starch and sustains the assimilate transport to economically important plant organs such as fruit, tubers and storage roots. Thus, autophagy as a process directly affecting stress resistance, senescence and translocation of water and assimilates, represents a potentially very important target for regulation of plant functions, which thus far has not been used for generation of new crop varieties or in other applications in agriculture. The review discusses the structural types of autophagy (S. Reumann et al., 2010), molecular pathways of autophagy regulation (F. Reggiori et al., 2013) and cellular mechanisms of assembly of autophagic machinery, fo-

cusing on their potential use in agricultural technologies (Y.-Y. Chang et al., 2009; S. Han et al., 2015), first of all, to counterpart the deleterious effects of abiotic stress factors.

Keywords: autophagy, potassium, programmed cell death, senescence, stress, assimilate transport, crop yield

Plants overcome adverse environmental effects (drought, salinization, drastic changes in temperature, etc.) without having any possibility to physically avoid them. Annually, a significant part of the crop yield is lost in the world due to adverse environmental factors. There is an acute need to create technologies providing an increase in the resistance of plants, first of all, important agricultural crops, to abiotic and biotic stresses. One of the targets of directed selection for stress-resistant agricultural crop plants may be the autophagy process providing the survival of adverse environmental conditions by the plant at a cellular level.

Autophagy is an intracellular process, resulting in the removal of damaged sub-cellular structures, renovation of organelles and recycling of macromolecules [1-3]. During autophagy, cellular components are subjected to degradation in acidic lytic compartments, and the released low-molecular-weight compounds and energy are used for building new structures. Autophagy is inherent to all types of eukaryotic cells and is an ancient, evolutionary highly conservative catabolic program; however, its mechanisms in animal, yeast and plant cells are different [3]. Thereby, it should be noted that research on autophagy in plants significantly lags behind the studies of this process in animals and yeasts.

The processes of programmed cell death (PCD) in plants have also been studied to a significantly less extent than those in animals. There is still no significantly clear morphological classification of PCD in plant cells. In contrast to animals, it is uncommon to refer to apoptosis in plants because the features of cellular organization of plants exclude the manifestation of a number of morphological features characteristic for this type of PCD [4], although there are mentions of an apoptosis-like pathway and the formation of apoptosis-like bodies in plant cells [5]. According to one classification, there are two main types of cell death in plants, vacuolar and necrotic [4]. It is known that PCD in plants occurs at the renovation of root cap cells, elimination of cells in the endosperm aleuronic layer at the completion of seed sprouting, providing the growth of pollen tube to the embryo sac, the formation of xylem vessels and phloem sieve tubes [6-8]. At the same time, both programs, autophagy and PCD, are an important part of the response to stress.

In the present review, the authors will discuss how autophagy occurs in plants, what its main functions are in the plant organism in the absence of stress, and also assess the role of autophagy in the stress response: its cytoprotective function and participation in the starting stages of development of vacuolar programmed cell death. In connection with the identified role of cytoplasmic potassium as one of the crucial regulators of plant response to stress, including the triggering of the autophagy and PCD programs, the components of regulation of the amount of cytoplasmic potassium have been reviewed for the determination of potential targets for increasing plant stress resistance.

Role of autophagy in physiological processes. In plant cells, as well as in animal and fungal cells, damaged (used and oxidized) proteins or those which are required to the cell no more are removed via autophagy. In contrast to the proteasome degradation system responsible for the removal of short-living proteins, the autophagy process enables the cell to remove long-lived proteins [9]. Moreover, autophagy is involved in degradation of entire cellular organelles. It was initially found that autophagy is induced in response to stress factors, in which connection it was believed that its role consists predominantly in

adaptation to adverse conditions [10, 11]. However, as it was found later, autophagy (basal or constitutive) also occurs in the absence of stress effects and serves as one of the key factors of maintaining cell vitality [3, 7, 12, 13].

Constitutive autophagy is necessary for maintaining homeostasis at a cellular level because proteins in the cell are inevitably oxidized during metabolic reactions, and also by air oxygen. The plants mutant in autophagy genes and incapable of carrying out this process are susceptible to early aging even under beneficial conditions [7]. Moreover, basal autophagy provides for the replenishment of the pool of amino acids and other nutrients required by the cell as a building material for carrying out anabolic reactions.

It was shown that autophagy is involved in the plant development processes. Lytic cleavage of starch and reserve proteins contained in seeds during sprouting of the latter occurs at its involvement [14]. The formed low-molecular-weight compounds (sugars and amino acids) are transported to the cells of forming organs. At maturation of seeds, nutrients obtained as a result of autophagic degradation of proteins in aging leaves may be delivered thereto [7, 15]. However, no significant disorders in development were observed under normal conditions in most mutants in the *atg* gene incapable of carrying out autophagy. This allows making a conclusion that constitutive autophagy does not play a significant role in growth processes and plant development in the absence of stress. On the contrary, increased sensitivity of such mutant to carbon and nitrogen deficiency and also to other stress conditions was established [3, 16].

Nocturnal autophagy was found relatively recently. It was found that mutants of *Arabidopsis* and tobacco in the specific autophagy genes (autophagy-related genes, *ATG*) are incapable of recycling starch overnight, accumulated in leaves during daily photosynthesis [17]. Treatment with autophagy inhibitors has led to the same effect. As a result of thorough cytological studies in the mesophyllic cells of the wild-type plants, bodies were found that contain starch, which were subjected to degradation in vacuoles. These bodies were not present in the cells of the plants incapable of autophagy due to genetic defects in the *atg* genes or due to exposure to inhibitors. The authors have suggested that enzymes catalyzing starch breakage are partly localized in lysosomes and at the nocturnal breakup of leave starch the bodies are first gemmated from chloroplasts which are then subjected to degradation according to the autophagy mechanism [17].

However, the most important role in plants is played by the so-called stress-induced autophagy. The activation of this autophagy type is very often associated with the production of active oxygen forms [18, 19]. Activation of autophagy in root cells of higher plants occurred in response to salinization, hypoxia and reaeration, water deficiency, treatment with oxidizers, gene-toxic agents and ionization radiation [18]. The leading role of autophagy in the immune response of plants was established. It facilitates the development of hyper-sensitivity reaction in response to the attack of necrotrophs or non-virulent biotrophs, but thus limits its spontaneous uncontrolled expansion. Autophagy also enhances the resistance of plants to biotrophs and necrotrophs based on the salicylate and jasmonate signaling system, participates in virus-induced gene silencing processes [20].

In this review, the authors will concentrate on the role of autophagy in plant resistance to abiotic stresses, which currently cause maximum harm to the productivity of agricultural crops compared to other stress types.

Structural types of autophagy. It was originally believed that autophagy is a non-specific pathway of cell component degradation. It is the non-specific mass degradation of various cellular structures simultaneously according to the autophagy mechanism that is activated in plants at nitrogen and carbon deficiency [3]. However, currently it has been convincingly proven that

autophagy may be highly selective, and autophagy types have been described that are highly specific to certain organelles: mitochondria (mitophagy) [21], chloroplasts (chlorophagy) [22], peroxisomes (pexophagy) [13, 23], ribosomes [24]. The selectivity is achieved involving receptor proteins specific to the particular organelles [7, 25].

Depending on the cytological mechanism, two structural types of autophagy may be identified: micro- and macroautophagy. At microautophagy, the delivery of cytoplasmic components to acidic lytic compartments (vacuoles in plant cells) occurs due to membrane invagination [3, 26]. Such type is activated, for example, at seed sprouting [14, 27]. The cytological markers of autophagy are double-membrane organelles called autophagosomes. The formation of autophagosomes begins with the formation of a preautophagosomal structure (also called a phagophore assemble site, PAS) around sub-cellular particles. Further, the growth of this structure occurs, which leads to the formation of a closed double membrane around the components to be recycled, after which their delivery to the place of degradation occurs (to a central vacuole of plant and yeast cells or to lysosomes of animal cells) [7, 22, 26]. In plants, autophagosomes first merge with lysosomes containing acidic lytic enzymes, their internal compartment being acidified, and autolysosomes are formed. Then the outer membrane of the autolysosome merges with tonoplast, and the partially degraded content of the autolysosome surrounded by one membrane (autophagy body) enters the vacuole [7]. Often, exactly this type of macroautophagy is meant as "autophagy" (Fig. 1).

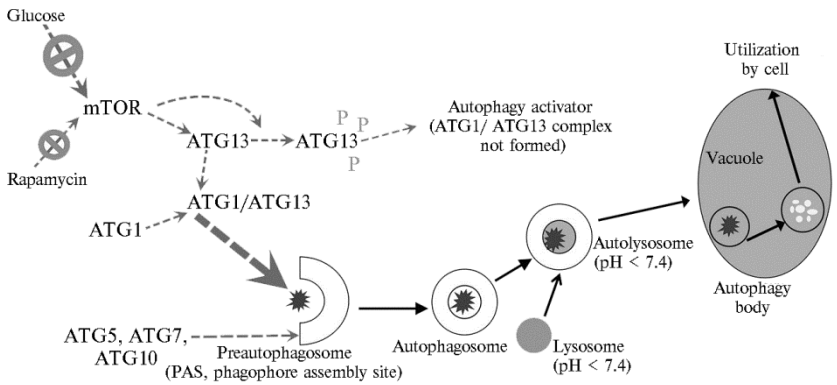

Fig. 1. Main organelles and proteins providing for inducing and progress of macroautophagy: mTOR — TOR-kinase; rapamycin, glucose — TOR-kinase inhibitors; ATG1, ATG5, ATG7, ATG10, ATG13 — component proteins of main autophagy complexes. Dashed arrows represent signal processes; black arrows represent the sequence of events at the level of sub-cellular structures.

Both structural types of autophagy were described in plants as well [10, 11, 14, 28]. In animal cells, apart from these, the third type of autophagy is known: chaperone-dependent autophagy. In its mechanism, chaperone proteins of the HSP family (heat shock proteins) are involved, which bind to the damaged proteins and deliver them to the lysosomal membrane [26]. In plants and yeasts, the Cvt pathway (cytoplasm-to-vacuole targeting) functions similarly; it is used for transporting the precursors of lytic enzymes to the vacuole [3]. Therefore, the Cvt mechanism is one of the selective types of autophagy, but it is more related to biosynthesis processes, not degradation processes [29]. Moreover, there are notes that autophagy is involved in the biosynthesis of the central vacuole. As a whole, there are more and more recent publications indicating that autophagic proteins and structural components, apart from carrying out the degradation of cellular components, may be involved in the circulation of cellular membranes,

including endo- and exocytosis [30, 31].

Molecular and genetic basis and mechanisms of autophagy development in plants. The genes encoding the protein components of the autophagy pathway (*ATG*) are highly conservative and are represented in all groups of eukaryotic organisms. Originally, the autophagy mechanism was discovered using the yeast model of *Saccharomyces cerevisiae*, and currently, about 40 *ATG*-genes are described for yeasts [25]. Most homologs of the *ATG*-genes were found in the plants as well [3]. Thereby, entire gene families in *A. thaliana* correspond to some single autophagic genes of *S. cerevisiae*. For example, homologs of *ATG12*, *ATG13*, *ATG8*, *ATG4* and *ATG18* are represented by several genes [32].

ATG proteins are classified in four groups involved at different stages of autophagy: ATG1-kinase complex (comprises ATG1, ATG13, ATG11, ATG17, ATG29, ATG31, ATG101); phosphatidylinositol-3-(PI_3)-kinase complex (VPS34, VPS15, ATG6, ATG14, ATG15, ATG38); ATG9-complex (ATG9, ATG2, ATG23, ATG27, ATG18); two Ubiquitin-like conjugation systems comprising complex 1 (ATG12, ATG5, ATG7, ATG10, ATG16) and complex 2 (ATG8, ATG4, ATG7, ATG3) [1, 3, 33]. Five main stages of autophagy may be identified: induction, formation of a preautophagosomal structure, maturation and expansion of an autophagosome, docking and merging with tonoplast, degradation of the autophagy body [2].

The key structure in the induction of autophagy is the kinase complex ATG1/ATG13 [34, 35]. The auxiliary proteins ATG17 and ATG11 participate in its formation. Their homologs have been identified in plants only recently [33, 36]. The formation of the preautophagosomal structure is initiated by binding of ATG17 to ATG29 and ATG31 [34, 37]. ATG1 binds to ATG17-ATG-29-ATG31 one of the first. Its binding to the three-component complex is mediated by the ATG13 protein, which has binding sites for both ATG1 and ATG17. These interactions facilitate an increase in the kinase activity of ATG1, providing for the addition of other proteins of the initiator complex. It has been recently discovered that ATG13 may facilitate the formation of dimers of the ATG1 protein, due to which the activation of this kinase according to the positive feedback principle is possible [34]. As a result of complex information interactions between ATG17 and ATG1, the initiator complex ATG1-ATG13-ATG17-ATG29-ATG31 (a scaffold of the newly formed autophagosome) emerges [3].

The next stage of autophagy is the growth or expansion of the autophagosome. For this purpose, the presence of phosphatidylinositol-3-phosphate (PI_3P) is necessary, which integrates into the membrane of the autophagosome. The amount of this phospholipid serves as another factor controlling the triggering of autophagy. Its content depends on the activity of the antagonist enzymes, phosphatidylinositol-3-kinases (PI_3K) and PI_3P-phosphatases. PI_3P is formed due to the activity of the PI_3-kinase complex 1 (PI_3K 1). It comprises the following proteins: VPS34 (vacuolar protein sorting-associated protein 34) that is related to class III phosphatidylinositol-3-kinases and plays a role of a catalytic sub-unit in the complex; VPS15 that serves as the activator sub-unit of the complex and anchors it in the autophagosomal membrane; ATG6 (homolog of mammal Beclin-1) [37]. The latter plays an important regulatory role: in animal cells, the binding of Bcl-2 and Bcl-1 serves as one of the key stages of autophagy initiation. However, no homolog of Bcl-2 was found in plants. In yeasts and mammals, another component of the PI_3K-complex is known — ATG14. There is still no data about the discovery of this protein in plant organisms in the literature and the GenBank database (NCBI) [33, 38]. Following from its important function, it is suggested that it must be present in plant cells [38].

Further, the assembly of conjugation complexes of Ubiquitin-like proteins begins. It is believed that the first event is the binding of ATG12 to ATG7. ATG7 has an E1-like activating ability and is required for the assembly of both complexes [39, 40]. Then ATG10, exhibiting E2-like conjugating activity, attaches to ATG7-ATG12. These enzymes perform reactions required for forming a bond between the ATG12 and ATG5 proteins [20, 40]. In order to bind the ATG12-ATG5 conjugate with phagophore, another protein is required, ATG16 [40, 41].

The main protein of the second complex is ATG8, an important regulator of autophagosome growth and formation. ATG8 is a small (14 kDa) ubiquitin-like protein. It is synthesized in a form of precursor and is subjected to significant post-translation modifications [42]. In the processing of ATG8, a redox-controlled enzyme, cysteine-dependent protease ATG4 is involved [43, 44]. Due to the cleavage of the amino acid sequence from the C-terminus of ATG8, its binding to the amino group of phosphatidylethanolamine (PE) becomes possible, which provides for the anchoring of the ATG8 protein in the autophagosomal membrane [45]. The E1-like enzyme ATG7 and E2-like enzyme ATG3 are responsible for the activation of ATG8 and the attachment of PE [41, 43]. Further, both complexes interact and the covalent binding of proteins of the second complex, ATG8 and ATG12 occurs via the protein of the first complex, ATG5, having E3-like ATG8-ligase activity. ATG12-ATG5 is involved in the transfer of ATG8 to the phagophore [46]. The lipids required for the further growth of the autophagosome are supplied from endoplasmic reticulum via the protein complex based on ATG9 [47]. The transfer of autophagosomes and autolysosomes in the cytosol is carried out with the mediation of cytoskeleton elements [48, 49]. Merging of autophagosomal membranes with lysosomes and with tonoplast occurs with the participation of the SNARE proteins [50].

Regulation of autophagy at the molecular level. At the present time, in plants two key regulators (inhibitors) of autophagy have been found that react to the concentration of nutrients: TOR-kinase [9] and the cytosolic isoform of the glyceraldehyde-3-phosphate dehydrogenase enzyme (GAPDH) [51, 52].

TOR-kinase (mTOR, the mammalian/mechanistic target of rapamycin) is a highly conservative serine-threonine protein kinase in eukaryotes, the most important activator of anabolism and the suppressor of catabolism in the cell [3]. TOR-kinase serves as a regulator for stress-induced autophagy, associated in the first place with an insufficient supply of carbon and nitrogen in the cell. In 2005, the dependence of the autophagy processes on the activity of TOR-kinase in the single-cell alga *Chlamydomonas reinhardtii* was confirmed [53]. It was proven in the paper by Liu *et al.* [54] that a decrease in TOR activity induces autophagy in a plant cell.

The blocker of TOR-kinase is rapamycin, an antibiotic of bacterial origin, synthesized by soil bacterium *Streptomyces hygroscopicus* [55]. It was reported earlier that despite the regulation of this process by Tor, the plants, in contrast to yeasts and animals, are not sensitive to rapamycin [54]. It was then established that rapamycin exerts an inhibitory action on the plant TOR-kinase; however, only in concentrations higher than the one in case of animal cells [9]. According to Xiong *et al.* [9], the concentrations, at which the rapamycin effect was manifested in plant cells, are 100-1000 nM, whereas in animal ones these are 10-50 nM. The presence of rapamycin in the said concentrations decreases TOR activity, which is morphologically manifested in slowing the root growth in *Arabidopsis thaliana* [9].

The key mediator in the induction of autophagy in response to stress is

the ATG13 protein [3, 35, 36, 56]. Under normal physiological conditions, the TOR-kinase phosphorylates ATG13. Such hyper-phosphorylated form of ATG13 has low affinity to ATG1, and the ATG1/ATG13 complex that initiates the formation of autophagosome is not formed. Binding of ATG1/ATG13 only becomes possible at a decrease in the activity of the TOR-kinase [3, 34, 56]. A deficiency of nutrients in the cell becomes a signal inhibiting the phosphorylation cascade of PI3K/TOR kinases, and results in a decrease in the activity of the TOR-kinase [54]. I.e. stress caused by carbon or nitrogen deficiency initiates autophagy (see Fig. 1).

Recently, another autophagy inhibitor was found in plants: glyceraldehyde-3-phosphate dehydrogenase enzyme (GAPDH) [51, 52]. The *Arabidopsis* forms deficient in the cytosolic isoform of this enzyme demonstrated enhancement of constitutive autophagy and also a high degree of oxidative stress and activation of PCD [52]. Production of ROS by cells in response to the pathogen attack was, on the contrary, decreased in such plants [52]. It was proved by the example of tobacco cells that GAPDH directly interacts with the component of the second system of ubiquitin-like conjugation, the ATG3 protein, suppressing its function; the inhibition is removed upon exposure to ROS [51]. Therefore, GAPDH, as well as the TOR-kinase, provide a direct relationship between the metabolic status of the cell and induction of autophagy, but this relationship is under redox control.

In yeasts and mammals, the important regulators of autophagy are kinases: AMPK (AMP-activated protein kinase) in mammals, SNF1 (sucrose non-fermenting 1) in yeasts [57]. They react to the alteration of energy charge, which is described as

$$([ATP] + {}^1/_2[ADP])/([ATP] + [ADP] + [AMP]),$$

and activate autophagy (directly or by inhibiting TOR-kinases). Several homologs of SNF1/AMPK are known in plants. For one of them (the KIN10 kinase in *Arabidopsis*), a role of autophagy activator has been recently shown under deficiency, hypoxia and water deficiency conditions [58].

Relationship of autophagy and programmed cell death. The role of autophagy in the development of PCD is ambiguous [15, 27, 59]. On the one hand, autophagy may serve as a method of avoiding cell death, and the cytoprotective function of autophagy is associated with this [60, 61]. On the other hand, activation of autophagy in some conditions precedes triggering of cell death programs, and in this case, autophagy is one of the starting stages of PCD [62]. Thus, in the process of vacuolar cell death, a decrease in the volume of the cytoplasm and an increase in the volume occupied by vacuoles are observed. These events are accompanied by the enhancement of autophagy and the rupture of tonoplast, accompanied by the release of hydrolases, which leads to the destruction of protoplast. Up to the moment of tonoplast rupture, the integrity of the plasmatic membrane, mitochondrial membranes and those of other organelles is maintained [4]. The entire process takes, as a rule, a long time: up to several days [63]. In contrast to vacuolar death, necrotic death develops much more rapidly and is characterized by the shrinkage of the protoplasm, early destruction of the plasmatic membrane and membrane organelles, the disruption of mitochondrial functioning and the accompanied accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the cytoplasm [63, 64].

The cytoprotective role of autophagy is demonstrated by studies of knock-out mutants, in cells of which its development is impossible. The insertion mutants of *A. thaliana atg5-1* [46] and *atg7-1* [39] are characterized at a long photoperiod by normal growth and development. However, at a short photoperiod, the mutants of both lines grow slower, have less seed productivity and

are subject to premature aging compared to the wild type. Moreover, they have elevated sensitivity to stress, especially to the deficiency of microelements. As a whole, these plants exhibit lesser viability and have a significantly lower survivability rate compared to the wild type, beginning from 10-day growth in a medium with decreased nitrogen content. At conditioning in darkness, the survivability decreases in *atg5-1* on the 2nd day already and that in *atg7-1* on the 4th day, whereas in natural ecotypes it is correspondingly on the 6th and 8th day [39, 46]. The *atg13* mutation in *A. thaliana* is phenotypically characterized in several lines [35]. They are differently susceptible to early aging under short day conditions. In the nitrogen-deficient medium, the growth of such sprouts is slower compared to the wild type. Chlorophyll synthesis in the leaves is disrupted. They are also more sensitive to carbon deficiency in the medium. Darkening for 10 hours does not affect these lines so strongly as the plants with the disrupted formation of ubiquitin-like conjugation complexes. However, already after 13 hours of conditioning in darkness, a marked difference in stability is found between the *atg13* plants and wild types, especially in double mutants [35]. The *atg10* mutants [41] were hyper-sensitive to carbon and nitrogen deficiency, and also demonstrated spontaneous development of PCD.

In the cells of plant roots upon exposure to abiotic stresses leading to the development of PCD, the autophagy symptoms are often observed [18]. It can be hypothesized that it is originally activated as a cytoprotective mechanism. But after passing through the "point of no return" it becomes a necessary stage of PCD development.

Hypothesis of potassium regulation of autophagy and programmed cell death. From the mid-1980s, in plant biology, the concepts of control and coordination of physiological reactions at stress via cytoplasmic Ca^{2+} and reactive oxygen species (ROS) are actively developed [65, 66]. It is known that the generation of ROS plays a significant role in the regulation of cellular metabolism. ROS are inevitably formed at redox reactions in the cell both under normal conditions and upon exposure to stressors (exposure to pathogens, drought, salinization). The discovery of elevated synthesis of ROS at the early stages of stress response was the beginning of studies dedicated to functions of these molecules. One of such functions is the regulation of the activity of ion channels [67, 68].

The rapid release of K^+ is related to events accompanying reaction to stress in the plant cell. In the recent years, the theory about the participation of potassium in plant response to stress was developed [18, 68-70]. Potassium is the most abundant metal and cation in the plant cell. Its content by dry weight is 3-10%; therefore, the deficiency of this metal extremely adversely affects productivity. Adequate potassium supply is the basis of high yield and resistance of plants to stress effects. Being an irreplaceable macroelement, comprised in the vital NPK (Nitrogen-Phosphorus-Potassium) triplet, potassium plays key functions in plant life. In particular, it is responsible for the water balance and hydroskeleton of the cell, transpiration, closure of air pores and stretching growth. The trans-membrane streams of potassium form the diffusion membrane potential on the plasmatic membrane, tonoplast and endomembranes, which serves as a basis for a high difference of potentials on these membranes. Also, potassium plays a role of a non-specific activator of dozens of crucial anabolic enzymes of the cytoplasm [71]. Possibly, it stabilizes the low activity of proteases and nucleases, preventing unplanned triggering of autophagy and PCD [18, 69].

The potential-dependent potassium channels and also several nonselective cation channels (NSCC) are involved in potassium release at stress and during some development processes [68, 69]. It is important to note that the slow re-

lease of K^+ occurs under normal conditions as well [70]. Moreover, it is necessary for carrying out important physiological processes, for example, air pore regulation of transpiration [72-73]. The increase in potassium release at salt stress was shown long ago [74]. It was established that the release of K^+ is mostly mediated by depolarization-activated outwardly-rectifying K^+-channels [75]. The detailed mechanism of this process and its effect on further events in the cell as well are still to be discovered.

The outwardly-rectifying K^+-channels providing for Goldman rectification of the outgoing potassium current are activated at the depolarization of the plasmatic membrane and are related to the Shaker type. These are usually homo- or heterotetramers [70]. Each subunit comprises six trans-membrane domains, a pore domain, and a voltage sensor. At the assembly of a tetramer, the pore domains containing a specific amino acid sequence (GYGD) are combined in such a way that four potassium-binding sites appear inside the pore, i.e. a selective filter is formed [76]. In the plasmatic membrane of root cells of *A. thaliana*, two types of outwardly-rectifying Shaker channels are synthesized: SKOR (STELAR K^+-outward-rectifier) and GORK (guard cell outward-rectifying K^+-channel). Thereby, the SKOR-type channels are represented in parenchymal cells and mediate potassium current in the xylem vessels, whereas GORK are predominant in epidermal cells and are involved in potassium release from the root [70]. Both of these types are directly activated by ROS [77]. Channel opening is induced via the ROS-sensitive site in the molecular structure. In the case of SKOR, the role of the ROS sensor is played by the cysteine residue (Cys168) in the peptide sequence of the S3 domain in the contents of the potential-sensitive complex S1-S4 [77]. Due to the fact that structurally similar GORK and SKOR are significantly similar, it is suggested that in GORK the ROS-dependent activation is provided in the same way [18].

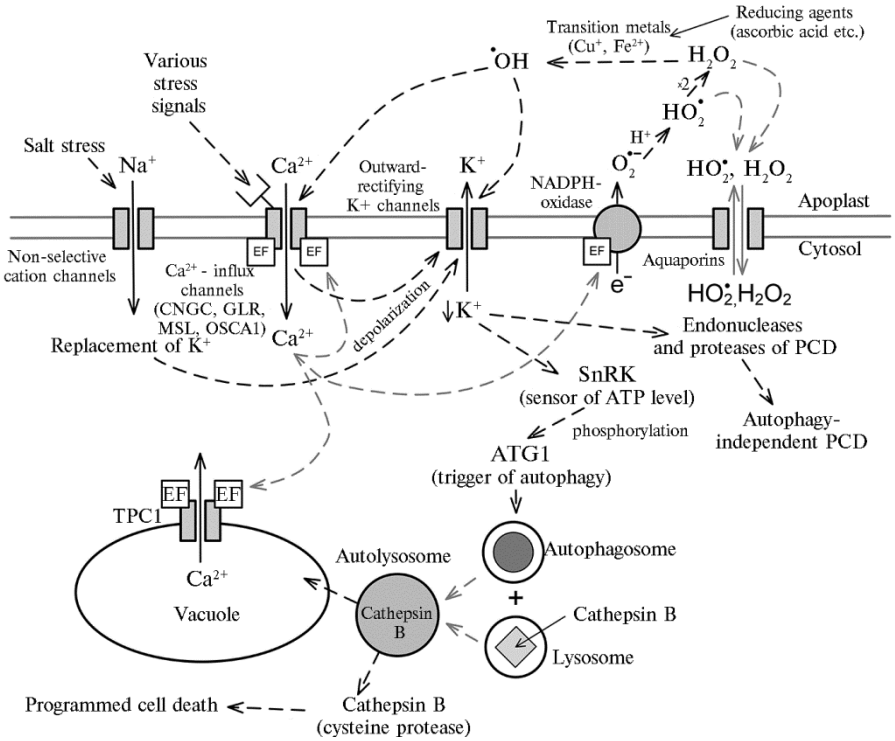

Fig. 2. Scheme of processes that are a basis of stress-induced autophagy and programmed cell death (PCD). The stress signals interact with specific receptors on the cell surface, which causes the depo-

larization of the plasmatic membrane, an increase in the cytoplasmic activity of calcium, an increase in the production of reactive oxygen species (ROS) due to the calcium-dependent activation of NADPH-oxidase. Depolarization also leads to the activation of the outward-rectifying potassium channels, which is further stimulated by ROS. A drastic drop in the concentration of cytoplasmic potassium leads to triggering of the autophagy and PCD reactions. ROS are also produced intracellularly and are transported into apoplast via aquaporins. The redox processes in the apoplast are controlled by the content of reduced transition metals and ascorbate (according to 18, with alterations). EF is EF-hand (protein domain); TPC1 is a two-port calcium channel. The grey arrows indicate secondary processes.

The activity of the GORK channel is stimulated by hydroxyl radicals ($^{\bullet}OH$), which are generated by the Ca^{2+}-dependent NADPH-oxidases at reaction almost to all types of stress, including salinization, drought, pathogen attack, etc. A decrease in the potassium concentration in the cytoplasm, in its turn, stimulates the activity of proteolytic enzymes, including caspase-like proteases, which play an important role in the PCD triggering mechanism in plants [45, 69, 70].

In a general form, the mechanism of developing a stress reaction involving the outgoing K^+ current in epidermal root cells may be represented as follows. At binding of the stress signal or pathogen elicitor with the plasmatic membrane receptor and also as a result of the supply of Na^+ via non-selective channels, the activation of Ca^{2+}-permeable cation channels occurs, which leads to an increase in the Ca^{2+} concentration in the cytoplasm. Calcium activates the NADPH-oxidase by binding to its cytoplasmic domain. Also, the Ca^{2+}-dependent activation of endonucleases and proteases may be observed [64]. NADPH-oxidase generates superoxide-anions ($O_2^{\bullet-}$), which react with protons, forming a hydroperoxide radical ($HO_2^{\bullet}$). Dismutation of $HO_2^{\bullet}$ gives hydrogen peroxide (H_2O_2), which becomes a source of oxygen for the synthesis of hydroxyl radicals ($^{\bullet}OH$) in Haber-Weiss reactions [78]. Peroxide and $^{\bullet}OH$ further activate the SKOR, GORK and Ca^{2+}-permeable channels, enhancing the supply of Ca^{2+} into the cytoplasm, and, in their turn, also stimulating NADPH-oxidase (Fig. 2). A positive feedback mechanism works, which may be stopped by systems for pumping Ca^{2+} away from the cytoplasm [79]. The membrane potential is restored by potassium release and due to an increase in the activity of the electrogenic H^+-ATPase pump. If membrane repolarization and restoration of the cellular potassium amount do not occur, the potassium-regulated plant proteases and endonucleases are activated, and the result is the initiation of the molecular PCD mechanism [70].

In the late 1990s, it was discovered that in mammal cells the decrease in the concentration of K^+ (together with the intake of Ca^{2+}) plays an important role in the apoptosis triggering mechanism [80, 81]. Potassium is a direct inhibitor of caspases, and its release from the cell activates these enzymes [82, 83]. Most probably, the similar mechanism exists in plant cells as well [18, 69]. According to the recent hypothesis, potassium in plants (depending on its content in cytoplasm) serves as a metabolism trigger, and the stress-induced release of K^+ may be a trigger of stopping the cell growth, inhibiting the biosynthesis, activation of catabolism and at strong stress at longer perspective, triggering the PCD [18]. An important step in developing this hypothesis is to test the potential stimulation of autophagy at the loss of K^+ by plant cells. It is still unknown whether autophagy is a K^+-dependent process in plants. It was shown that in *A. thaliana gork 1-1* mutants (in contrast to the natural ecotype Ws-0) at conditioning the roots in NaCl solution, the drastic accumulation of autophagosomes does not occur. The obtained data indicate that potassium loss plays a direct role in inducing autophagy [18].

Perspectives of studying autophagy. According to modern

concepts, autophagy plays a significant role in cell metabolism providing the renovation of cellular structures, the cleavage of damaged molecules and production therefrom of organic compounds required for the extraction and accumulation of energy. I.e. this effect is directed to cell survival. Autophagy is especially important at adaptation to various stress effects. It mainly defines the survival of plants under adverse and changing conditions. Despite the fact that autophagy is studied in sufficient detail, there are still many unresolved issues. In particular, it is not quite clear whether the rapid release of potassium ions from the cell always directly triggers the molecular autophagy mechanism (similarly to its induction of programmed cell death). The same concerns the relationship of autophagy with other intracellular processes. It was shown that the absence of autophagy adversely affects the endurance of plants to stress, that it influences biomass accumulation and seed productivity. However, the effect of autophagy is not so unambiguous because it is also involved in cell death processes. Nevertheless, these processes are an intrinsic part of organism development; they are necessary for the formation of many structures and passing of all life cycle stages.

In case the hypothesis that the regulatory cellular signal for triggering autophagy is a drastic decrease in the cytoplasmic potassium concentration is confirmed, its functions can appear to be even more significant, especially under conditions when the plant is exposed to adverse environmental factors. The directed manipulation with the degree of cellular component activity for maintaining the required potassium concentration in the cytoplasm at stress may be used at creation of stress-resistant plants, for decreasing their deaths and supporting growth processes under adverse environmental conditions.

Therefore, autophagy as a process directly relevant to the mechanisms of stress resistance, aging and to the transport of assimilates is an important potential target for regulation, which has not yet been used at creation of new cultivars and in practical applications in agriculture. Autophagy plays a double role: on the one hand, it is directed to the survival of the cell; on the other hand, it serves as part of the cell death process. In both cases, autophagy directly affects the development of plant organisms. In view of this, further studying of autophagy regulations, especially, more detailed disclosure of induction mechanisms of this process appears to be perspective.

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