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Molecular basis of phosphoinositide-specific phospholipase C signaling pathways in plant cells

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In plants external stimulus can be perceived and amplified in the cells by functional signaling cascades. Phosphoinositide-specific phospholipase C is an enzyme shown to initiate and provide key events in the cellular responses to extracellular signals. Both substrate and products of phospholipase C are involved in the regulation of numerous processes in plant cells. In this review, we focused on molecular basis of the phosphoinositide-specific phospholipase C signaling pathways. The data analyzed will help to elucidate the mechanisms responsible for plant's ability to respond to a variety of biotic and abiotic stress signals.

Keywords: phosphoinositide-specific phospholipase C, signal transduction.

Introduction. Phosphoinositide-specific PLC (PLC) is a key enzyme of the phosphoinositide signal transduction in cells of bacteria, protozoa, plants and animals. It hydrolyses phosphatidylinositol 4, 5-biphosphate producing inositol trisphosphate and diacylglycerol [1]. In the plant PLC plays an important role in the diversity of physiological processes. It is activated in response to various environmental effectors such as salt [2, 3], cold [4, 5], osmotic stresses and drought. Moreover, the plant PLC is a component of signaling pathways of some phytohormones, for example, abscisic acid (ABA) [10, 11] and cytokinin [12].

Having the unique functions, both substrate and PLC products participate in the regulation of numerous processes [1, 13]. Inositol trisphosphate and hexakisphosphate (inositol trisphosphate transmutation product) cause Ca^{2+} release from the intracellular storage [14, 15]. Diacylglycerol can be phosphorylated by the diacylglycerol kinase producing the lipid messenger, phosphatidic acid. The vesicle transport in a cell is shown to be associated closely with intracellular localization, cyclization, motion and degradation of proteins, and regulated by the phospholipid molecules including phosphatidylinositol 4, 5-biphosphate and phosphatidic acid [16, 1 7].

Study on PLC activity both *in vitro* and *in vivo* is complicated by the phosphatidylinositol 4, 5-biphosphate low content in the higher plant organisms, and registration of inositol trisphosphate with thin layer chromatography is seldom successful [18]. The inositol trisphosphate analysis is often performed with the Amersham TRK1000 test [19]. Investigation of

PLC signaling pathways is mostly based on application of the enzyme inhibitors including U73122 and its inactive analog U73343. However, according to some information, under the influence of U73122, the slow increasing of Ca^{2+} in a Ca^{2+}-free medium as well as blocking Ca^{2+}channels of *L*-type are observed [21]. PLC is also studied using transgenic plants. The experiments on stimulation or depression of the PLC gene expression, required to clarify the PLC functions in the plant organisms, have been much successful [22-24]. The study on PLC characteristics will assist in further comprehension of the mechanisms of phospholipid signaling pathways considering different aspects of plant growth and development.

PLC Molecular Structure. The five types (, , , and) are distinguished among the mammalian PLC. They contain PH-domain that is a basic sequence required for the attachment to plasma membrane, binding with substrate and catalysis. They also have EF-domain important for the enzyme activation, *X*- and *Y*-sequences representing the catalytic centre, and C2-domain ensuring interaction with Ca^{2+} and lipids [25, 26]. Such structure is typical for PLC -isoform while there are additional domains in other PLC families. The plant PLC is structurally identical to the smallest mammalian PLC -isoform of about 60-70 kD, which has two EF-hand sequences, *X*-, *Y*-, and C2-domains but not the PH-domain typical for the other PLC isoforms [27].

However, it should be noted that on the sequence level PLC is closer to the mammalian PLC -isoform [28, 29]. The plant PLC does not contain PH-domain, which is necessary for the interaction of the animal PLC with plasma membranes. PLC contains C2-Ca^{2+}/phospholipids -binding domain fixing the catalytic centre in correct position. This domain participates in the interaction with membrane but its presence is not sufficient for the enzyme to form catalytically active state and other PLC domains are required to associate with plasma membranes [26, 30].

PLC Genes Expression and Evolution. For the first time, DNA encoding for PLC was identified in animal cells in 1988 [31]. Seven years later the *Arabidopsis thaliana L.* [2] and soybean (*Glycine max L.*) PLCs were cloned and the presence of this enzyme protein was demonstrated in both plasma membrane and cytosol [32]. Nowadays, a lot of the active PLC coding genes are known and the PLC proteins were obtained from certain plants, for example, potato (*Solanum tuberosum L.*) [33], mungbean (*Vigna radiata L.*), maise (*Zea mays L.*), rice (*Oryza sativa L.*) [1], and petunia (*Petunia inflata L.*) [30].

In the *A. thaliana* genome there are 9 *AtPLC* genes [22, 29]. *AtPLC1* - *AtPLC5* genes encode for PLC the activity of which was proved *in vitro,* whereas the enzymatic activity of *AtPLC8* and *AtPLC9* products is hardly probable [22, 34]. *AtPLC6* and *AtPLC7* contain the domain required for the PLC activity and encode the active enzyme. Previously it was established that *AtPLC7* encodes incomplete and non-functional protein with molecular mass of 30 KD [22]. However, subsequently there was discovered the full-chain *AtPLC7* DNA, probably encoding the PLC functional protein [35].

The catalytic domains of all PLCs have the similar structure and oxidation-reduction catalysis mechanism. The conservatism of topology and elements of the active site indicate their origin from a single allied protein. The phylogenetic history of eukaryotic PLC probably includes several stages of chain elongation and gene duplication. The *AtPLC* gene family evolution seems to go through several phases. The gene tandem of *AtPLC8* and *AtPLC9* arose due to the local duplication on later stages. *AtPLC1*, *AtPLC4* and *AtPLC5* make up a group of genes combined from small DNA fragments. Probably, the precursor of *AtPLC4/AtPLC5* and *AtPLC1/AtPLC3* arose from a single gene during the duplication in the 5th chromosome with the further *AtPLC3* duplication and translocation onto the 4th chromosome responsible for the further gene distribution [35].

The expression of *AtPLC* gene increases in response to such environmental factors as dehydration, salinization and cold stress [2, 36]. The potato PLC genes are expressed in response to the wounding stress and water deficit [33].

The *AtPLC1* and *AtPLC6* transcription is induced in response to some abiotical stresses including dehydration, salinity and cold stress [2, 34] (see the table). The transcription level increment is supposed to result in the *AtPLC* protein increase and thereby it activates the signaling pathways of up- or down-regulation of genes participating in various cell reactions. For exam-

Enhancement of phosphatydylinositol specific phospholipase C expression in plant cells in response to abiotic factors and influence of abscisic acid.

Influence	Activated isoform	Object	Literature source
Abscisic acid	*AtPLC6*	*Arabidopsis thaliana* L.	[34]
	TfPLC2	*Torenia fournieri* L.	[85]
	AtPLC1–9, кроме *AtPLC2*	*A. thaliana* L.	[35]
Decrease of the temperature	*AtPLC1*	*A. thaliana* L.	[2]
	AtPLC1–5	*A. thaliana* L.	[22]
	BnPLC2	*Brassica napus* L.	[9]
	ZmPLC	*Zea mays* L.	[86]
	AtPLC1–9, кроме *AtPLC2*	*A. thaliana* L.	[35]
Increase of the temperature	*AtPLC6*	*A. thaliana* L.	[34]
Salt stress	*AtPLC1*	*A. thaliana* L.	[2]
	AtPLC1–5	*A. thaliana* L.	[22]
	AtPLC6	*A. thaliana* L.	[34]
	VrPLC3	*Vigna radiata* L.	[7]
	ZmPLC	*Z. mays* L.	[86]
	ZmPLC1	*Z. mays* L.	[38]
Dehydration	*AtPLC1*	*A. thaliana* L.	[2]
	StPLC1	*Solanum tuberosum* L.	[33]
	AtPLC1–5	*A. thaliana* L.	[22]
	VrPLC3	*V. radiata* L.	[7]
	ZmPLC	*Z. mays* L.	[86]
	TfPLC2	*T. fournieri* L.	[85]

ple, the *AtPLC1* transcription, the enzyme activation and increase in the inositol trisphosphate level were demonstrated to be required for the further gene expression in response to the abscisic acid influence.

AtPLC1 is activated in response to the influence of salt, abscisic acid, cold and dehydration. The *AtPLC2* gene expression is not induced under the influence of abiotical stresses [37]. Unlike the *AtPLC2*, the other 8 genes of *AtPLC* are induced in response to the external influence. For *AtPLC8* and *AtPLC9* the transcription level increases less than twofold under the influence of all external stimuli [35]. (More than two-fold intensification of) The *AtPLC6* induction rise more than twofold was demonstrated as a result of action of some external factors [2].

The physiological role of PLC in the response of plants to the abiotic stress was described using the transgenic maize. The abiotic stress does not change either the wild phenotype or the phenotype of plants with depressed or heightened PLC gene expression which were grown in the optimal conditions. However, under the influence of water stress the plants with diminished *PLC1* expression differed by a low water content in tissues, osmoregulation deterioration, photosynthetic activity decay, high percentage of ion loss, higher lipid peroxidation intensity and lesser productivity in com-

parison with the wild. There was drawn a conclusion on failure of signal transduction mechanism and, because of this, inability of cells of providing the adaptation to the stress which indicates the essential role of PLC genes in the regulation of response to the stress caused by water deficit [38].

PLC Kinetic Properties. Most of the active plant's PLCs were obtained from cytosol, but as the substrate of this enzyme is associated with membrane the enzyme activity in cytosol and membranes may differ. Because of this one of the most interesting tasks is to study the activation of PLC on the membrane surface. The PLC activity in *Catharanthus roseus L* was described using the lipid substrates, distributed in the phospholipid vesicles, phospholipid micelles and monolayer in the air-water medium [39]. The application of P^{33}-labeled substrate for the direct measurement of the Catharanthus *roseus L.* PLC activity revealed dependence of the monolayer PC-PS proportion and amount on the pressure. The PLC activity increases when the pressure is raised up to 20 mN/m^2 and reaches the maximum under these conditions. The further pressurization leads to the PLC activity decay. Probably, the PLC activity reduction is caused by decrease in the enzyme ability to bind the substrate. This phenomenon is specific and seems to depend on the phosphatidylinositol 4, 5-biphosphate concentration. These results differ from those received for the animal -PLC and are similar to -PLC. This is unexpected because structurally the plant PLC is closer to the -PLC isoform. However, it was demonstrated that the -PLC isoform activity decreases linearly at increasing surface pressure. What is the reason of the differences between the activities of the plant PLC and -PLC in a monolayer is unknown, but the dependence of different isoforms activity on the characteristics of the surfaces which they interact with was determined. Thus, as a result of studying the PLC of plants and other organisms [40, 41] it was established that the phosphatidylinositol 4,5-biphosphate hydrolysis in a monolayer depended on the surface pressure determining the peculiarities of interaction between the enzymes of this family and the lipid surface [39].

This information and the results, obtained using the monolayer substrate where the PLC activity decreased as the substrate pressure increased, indicate that PLC has to penetrate through the lipid aggregates in the case of the substrates binding and hydrolysis. The data on vesicular binding confirms the plant PLC interaction with the membrane surface by the phosphatidylinositol 4, 5-biphosphate substrate. To provide the formation of the PLC-mediated secondary messengers PLC can interact with the membrane surface in specific non-catalytic way with further binding or surface reorganization. This can promote a stable fixation of PLC on the membrane surface [42].

The unexpected results were received during investigation of the surface interacting site and further binding of the lipid substrate inside the membrane. Probably, the Michaelis-Menten equation curve with Hill coefficient close to 1 indicates the existence of a single binding site. The animal PLC amino-terminal region, containing pleckstrin homologous domain, is necessary for binding with phospholipid vesicles which contain phosphatidylinositol 4, 5-biphosphate. These results confirm that the presence of phosphatidylinositol 4, 5-biphosphate can be an important factor, required for the localization of the proteins, which contain this domain on the membrane surface. However, the pleckstrin homologous domain has not been revealed among the products of the plant PLC genes. Perhaps, the plant PLC initially has to affiliate with phosphatidylinositol 4, 5-biphosphate in the catalytic site n [43].

The enantiomer pure analogues of all natural PLC substrates, including both long- and short-chain phosphatidylinositol 4, 5-biphosphates, phosphatidylinositol 5-phosphates and non-phosphorylated phosphatidylinositols, were synthesised. The substrates phosphorylated at the 4-inositol bond (phosphatidylinositol 4, 5-biphosphate and phosphatidylinositol 5-phosphate) have very similar kinetic properties and their phosphorylation progresses 20-30 times more actively than those non-phosphorylated (phosphatidylinositol 4-phosphate and phosphoinositide). So it can be concluded that the interaction exactly with the 4-phosphate group is required for the PLC catalysis. Furthermore, the binding affinity of all four groups is rather similar that indicates the energy sufficiency of enzymatic binding with the 4-phosphate group for the catalysis.

The specificity of the wheat root PLC towards the polyphosphoinositides depends on the various factors:

pH (pH 6-7 - the phosphoinositide-phosphate hydrolysis, pH 6-6.5 - phosphoinositide 4, 5-biphosphate hydrolysis) and (kind of the) ions. The calcium, manganese and cobalt ions at the concentration of 4 mM diminish the PLC specificity towards phosphoinositide-biphosphate and intensify the phosphoinositide-phosphate hydrolysis. At high calcium concentration the PLC specificity changes in such row: phosphoinositide > phosphoinositide-4-phosphate > phosphoinositide 4, 5-biphosphate [45].

PLC can be divided into 2 groups by the *in vitro* properties: soluble forms which are specific towards phosphoinositide and require calcium of mM concentrations for catalysis, and PLCs, attached to the plasmalemma with a substrate specificity towards the polyphosphoinositides, requiring μM concentrations of calcium for activation (the optimum is 0.1-10 μM) [30]. The purified membrane PLC is highly specific towards phosphates (100% activity); crude enzyme is more specific towards the polyphosphoinositides (10% activity for phosphoinositide-phosphate and 30% for phosphoinositide-4, 5—biphosphate). The activity of purified form towards phosphoinositide 4, 5-biphosphate is restored by addition of a protein lost during the enzyme purification. However, it was demonstrated that this regulatory factor is not a G-protein [45].

Regulation of PLC activity. *G-proteins.* Heterotrimeric G-protein consists of -, - and -subunits and operates as a molecular switch controlling a great number of cellular reactions, transmitting the signal from the cell surface receptors to the intracellular elicitors, such as PLC, phospholipase D, cyclases, ion channels, phosphodiesterases, etc. [46]. In the human genome there are about 1000 genes for G-proteins, whereas in the *A. thaliana* genome only one gene of G-protein, participating in the cell cycle regulation [47] and abscisic acid signaling in the guard cells [48], has been found up to now. A G-protein was demonstrated to participate in the ion channel regulation [49] and cellular proliferation of the *A. thaliana* plants [50]. Moreover, the mutations in the *A. thaliana and* rice G-protein result in the damage of a wide range of processes, such as seed germination, spear and root growth, stoma movement [50].

Three legumes G-protein subunits were obtained and described; G-protein participation in the salt and thermal stress signal transduction, and the interaction between G-protein and PLC were demonstrated. The interaction between the G-protein -subunit is an important stage of salinization signal transduction. The thermotolerance is based on the G -mediated signal transduction. A further study is required to clarify the regulation of these proteins in response to the salt and thermal stresses, and to understand their role in the adaptation [51].

The change in inositol trisphosphate concentration is observed in response to an increase in the tyrosine kinase activity and PLC activation during the G-protein activation at infection of *Citrus limon L.* lesion with *Altemaria alternate* fungus. It was established that two signal pathways are activated: one – with participation of G-protein, another - with tyrosine kinase-dependent PLC. A possibility of the relation between PLC activation and tyrosine kinases requires further research [52].

The regulation of PLC activity by calcium ions. Calcium is a main activator among the ions able to influence the PLC activity. The optimum calcium concentration for the PLC activity in microsome fractions and plasma membranes of *Brassica napus* cells is 10^{-5}-10^{-4} M [53].

The maximum phosphoinositide 4, 5-biphosphate hydrolysis by the soya PLC is observed at pH optimum of 6.5-7.5 [54]. The lipid hydrolysis by the potato PLC intensified at the calcium concentration of 100 μM. The specificity of PLC 1 towards phosphoinositide 4, 5-biphosphate starts to diminish at more than 100 μM calcium, whereas PLC 2 and PLC 3 lose the specificity at a higher calcium concentration of 100-10000 μM [33].

The *A. thaliana* PLC 1 inhibition does not depress the *Cor* and *RD29a* stress genes expression induced by the abscisic acid [20]. Perhaps, the synthesis of the cyclic ADP-ribose will mediate the initial cytosolic calcium concentration rise resulting in the PLC activation. However, this does not rule out the possibility that other isoforms of PLC can initiate simultaneously a primary response and a calcium flux into the cell even at low calcium concentrations. At least one of the *A. thaliana* PLC isoforms, namely AtPLC4, is active in the absence of calcium [22].

AtPLC1 mainly hydrolyses phosphoinositide 4,5-biphosphate. At the optimum calcium concentration phosphoinositide 4, 5-biphosphate is hydrolyzed

by 100 times more efficiently than phosphoinositide. The maximum hydrolysis is observed at 1-50 μM calcium, and at the calcium concentration of more than 1 mM the phosphoinositide hydrolysis prevails (like the potato and soya PLC). The Michaelis-Menten constant for the PLC of *C. roses* roots is 0.0518 and the substrate constant is 45.5μM, the maximum reaction rate is 137.2 picomoles/min [39].

A need for Ca^{2+} for the activation of many plant PLC seem to indicate that inositol trisphosphate formation is not a primary response to the stress since the Ca^{2+} level rise is first required for the PLC activation and phosphoinositide formation. The calcium level can increase due to the calcium flux from extra cellular medium or because of the action of secondary messengers able to release Ca^{2+}, e.g. cyclic ADP-ribose [56, 57], nicotinic acid adeninedinucleotidphosphate [58], sphingosin-1-phosphate [59], hydrogen peroxide [60], and hexakisphosphate [61].

The study on regulation of five AtPLC isoforms by calcium demonstrated that *A. thaliana* PLC 2 is the most susceptible to the calcium ion effect: at the concentration of 10 nM its activity was 80% of maximum. The susceptibility to the μM calcium concentrations diminishes in the following order: PLC4 - PLC5 - PLC1. PLC 3 has the weakest response to calcium: at 10 nM concentration the activity is 15% of maximum. PLC4 is the most stable at low calcium level - its activity is 20% of maximum in the presence of EGTA. All *A. thaliana* PLCs except for PLC 3 are the most active at the calcium concentration of 3 μM and preserve their activity on the same level at the calcium concentration of 10 μM. In case of micro molar calcium concentrations the PLC isoform activities differ. PLC 2, PLC 4 and PLC 5 reach the maximum activity level at the calcium concentration of 1μM whereas PLC1 and PLC3 require higher calcium concentrations for the maximum activity. The overlapping of the *A. thaliana* various PLC genes expression indicates their functional excess. However, it is also possible that the regulation of each gene and PLC is exerted in different ways. The calcium release under the influence of one of the PLC isoforms can cause an activation of the others. The permanent expression of *A. thaliana* PLC2 and PLC3 evidences for their participation in the primary response to a stress, causing the calcium ion concentration rise to induce the PLC1, PLC4 and PLC4 genes expression along with other genes activated by calcium [22].

The Molecular Mechanisms of PLC Signal Pathway Realization in the Plant Cells. *Inositol triphosphate, hexakisphosphate.* The inositol trisphosphate administration into the cells results in the cytoplasmic calcium concentration rise, stomata closing, protoplasts swelling, fertilization tube growth inhibition, and plasmodesma closing [62]. Inositol trisphosphate is a secondary messenger which release calcium out of the intracellular depot in plant cells [14]. High-affinity sites for inositol trisphosphate binding in animals are located on the endoplasmic reticulum (ER). An assumed inositol trisphosphate receptor was obtained from the *Vigna radiate L* and characterised. In comparison with the animal cell receptors it is smaller (110 against 250 kD), and its activity is intensified by the inositol phosphate metabolites [63]. The existence of the calcium channels sensitive to inositol trisphosphate on the non-vacuolar membranes was proved. However, up to now the attempt to extract the inositol triphosphate-regulated channel protein has failed. The inositol trisphosphate receptors genes homologous to the animal ones also have not been found in the *A. thaliana* genome [64]. In the plants *Chenopodium rubrum L.* the inositol trisphosphate binding sites are located on the ER [54]. It was demonstrated that the inositide-2, 4, 5-trisphosphate stimulates calcium release from the intracellular depot rather efficiently, but slighter than inositide-1, 4, 5-trisphosphate [65]. Perhaps, the inositol triphosphate-phytase complex interacts with the inositol trisphosphate receptors resulting in calcium release from the microsome fractions whereas inotitide-1,3,4-trisphosphate does not have such capability since it does not bind to the receptors [65]. Study *in vitro* on the *Chenopodium Rubrum L* .plants revealed the interaction of inositol triphosphate-phytase with the inositol trisphosphate receptors, which requires the nanomolar concentration of the substrate. The inositol trisphosphate binding with a highly affine non-catalytic site of the phytase results in the essential conformation changes in the enzyme. This causes the formation of inositol triphosphate-phytase-receptor complex with much more active release of calcium comparing to free inositol trisphosphate [63]. These investigations are in

favour of the existence of a new signal cascade, regulating the calcium homeostasis, which is mediated by the hexakisphosphate-phytase system in the plant cells [65].

A role of hexakisphosphate is not studied enough. The *S. tuberosum L.* and *V. faba L.* stomata cells treatment with abscisic acid increases the level. The hexakisphosphate was shown to screen the inhibiting effect of abscisic acid and calcium on the potassium channels [15]. The hexakisphosphate and phosphatidic acid, formed in the yeast from inositol trisphosphate and diacylglycerol, regulate the gene transcription and mRNA transport [66]. This explains the absence of the genes, coding the calcium channels which are regulated by inositol triphosphate, and the absence of the protein kinase C genes in the genome of these organisms.

The inositol trisphosphate level is strictly regulated. The plant inositol phosphatases differ significantly from the animal ones. For the pants it was established *in vivo* [18] and *in vitro* [67] that the inositol-1, 4, 5-trisphosphate is hydrolysed by the enzymes inositol-polyphosphate-1'-phosphatase and inositol-polyphosphate-5'-phosphatase, to produce inositide-4, 5-biphosphate and inositide-1, 4-biphosphate.

Diacylglycerol, diacylglcerolpyrophosphate, phosphatidic acid. The increase in DAG level stimulates the H^+-ATP-activity and the stomata opening, affects cell division, hampers the protein movement through the plasmodesma and intensifies the phosphorylation [67]. The cell treatment with DAG *in vivo* results in the change of cytoskeleton flexibility the reduction of intervacuolar filament tension. DAG is also involved into the process of mitosis in the cells of stamen filaments. Furthermore, DAG induces the ion absorption in the isolated protoplasts of guard cells and stomata opening [62]. However, it is not established whether these effects are caused directly by DAG or its metabolites (for example, fatty acids and phosphatidic acid (PA)). Most of the observations indicate the DAG phosphorylation immediately after its formation [62, 69, and 70].

The phosphatidic acid rise was discovered in various types of the plant cells under the influence of the osmotic stress, wound, pathogens, abscisic acid, oxidative stress, Nod-factors, and drought [70]. The formation of 18:3/16:3- PA through the DAG phosphorylated by the DAG kinase was observed under the freezing and influence of the pathogens. The phospholipase D is also an important PA generator [1, 24].

There was discovered a lot of proteins able to bind with PA, for example, MARK [3] and protonic ATP [6], protein kinase influencing the actin polymerisation [71], NADPH-oxidase [72], protein kinase dependent on calcium [73], SNF-bound protein kinase SnRK2.10, regulative subunit of 2A protein phosphatase RCN1, DRG1, specific isoforms of the 14-3-3 protein GRF6 ( ) and GRF8 ( ), heat-shock protein isoforms, several tubulin isoforms, and the isoforms of phospho- enolpyruvate carboxylase (Ppc1 and Ppc3) [17, 71].

The PA signalling is always impetuous and transient that is ensured by the signal depression mechanism [70]. The restoration of the initial concentration is very important for most of the signal molecules. In the plant cells the PA signalling weakens due to its phosphorylation by PA kinase with producing diacylglcerolpyrophosphate (DGPF) [62, 70]. At rest the DGPF concentration in cells is very low, and the expression level of PA kinase, which is responsible for DGFP formation, remains constant for all plants. This stipulates a close association between the DGPF formation and its precursor PA availability. Since the DGPF formation coincides with PA level lowering, the PA kinase can take part in the PA signals depression. *In vivo* the PA kinase activation was discovered in yeast and in many plant systems in response to the various physiological stimuli, including his hyper osmotic stress, drought and pathogens attack. Thus, a possible role of DGPF as a secondary mediator for PLC signal trans- duction in the plant cells should not be excluded [70].

The Phosphatidylinositol 4, 5-biphosphate Functions in the Plant Cells. In the animal cells phosphatidylinositol 4, 5-biphosphate is not only a substrate of PLC, but it can be also a signaling molecule influencing various biological processes in cells [13]. The phosphatidylinositol 4, 5-biphosphate of higher plants is a regulator of the signal pathways. The change in intracellular phosphatidylinositol 4, 5-biphosphate level is observed in response to the influence of light, cold, gravitational stimulation, oxidative stress, G-proteins ac-

tivation, and pathogenic elicitors [74]. Possible processes with phosphatidylinositol 4, 5-biphosphate assistance are listed hereunder:

1. Ion channels regulation. Phosphatidylinositol 4, 5-biphosphate is considered to be a key regulator of ion channels activity, especially K^+ [75]. The phosphatidylinositol 4, 5-biphosphate accumulation in the plant plasma membrane induced by the salt stress can reflect this function [13].

2. Membranes formation and fluidity. During the mammalian cell division phosphatidylinositol 4, 5-biphosphate is mainly localized in the membrane and is required for the cytokinesis accomplishment for the active area formation, effective membrane fusion, and cell division [76, 77].

3. The cytoskeleton reorganisation. Phosphatidylinositol 4, 5-biphosphate is involved into the cleavage furrow formation. It is bound with actin-regulatory proteins and can influence their activity [78, 79, and 80]. Moreover, an increase in the local phosphatidylinositol 4, 5-biphosphate level can promote proteins interaction with the plasma membrane having specific phosphatidylinositol 4,5-biphosphate-binding sites [79, 81]. The plants are supposed to contain several such domains including the cytoskeleton organising proteins [1, 13]. The low phosphatidylinositol 4, 5-biphosphate level in plants can be explained by either a higher affinity of the plant protein domains towards lipids compared with the mammalian ones, or the affinity of complementary protein domains towards the other components, which are necessary for efficient binding with the plasma membrane. The confirmation of such functions may be the response observed during cell division and salt stress as well as changes in the cytoplasmic reticulum, induced by U71322, and an influence on the vacuoles morphology [13]. The phosphatidylinositol 4, 5-biphosphate participation in actin cytoskeleton regulation was also demonstrated during the fertilization tube growth [30].

4. The membrane transportation. In the mammalian cells a lot of polyphosphoinositides are involved into the exocytosis and/or endocytosis [79, 82]. However, the phosphatidylinositol 4, 5-biphosphate accumulation in small vesicle structures is not observed [84]. The phosphatidylinositol 4, 5-biphosphate polar gradient can reflect the membrane transportation events during the root fibrils growth [30, 84].

Despite the variety of PLC signalling mechanisms, a minor quantity of phosphatidylinositol 4, 5-biphosphate present in the membranes of higher plants is registered on the recently formed membranes of the dividing cells, accumulating in the membranes in response to the salt stress [13]. The decrease in phosphatidylinositol 4, 5-biphosphate level in the stomata guard cells in response to the abscisic acid influence indicates its participation in the abscisic acid signalling cascades, activating the stomata closing [55]. It was demonstrated that the phosphatidylinositol 4,5-biphosphate-binding protein inhibits the stomata opening induced by light, resulting in reduction of the free phosphatidylinositol 4,5-biphosphate level decrease in the inositol trisphosphate and phosphatidic acid formation under the influence of PLC and D; besides, it stops the stomata closing caused by abscisic acid [74]. Light-dependent increment of phosphatidylinositol 4,5-biphosphate content in the plasma membrane can occur not only due to increasing synthesis but also because of reduction of phosphatidylinositol 4,5-biphosphate hydrolysis by PLC. The diminished PLC expression reduces the inhibitory effect of abscisic acid on the seed germination and the influence of this phytohormone on the expression of the drought- and cold- responsive genes [37].

PLC plays a significant role in the abscisic acid signal transduction in the stomata guard cells. U73122 (a PLC inhibitor) reduces the guard cells response to the abscisic acid action and Ca^{2+} fluctuations in cytosol [11]. Moreover, a decrease in the PLC level in the stomata guard cells partially prevents the inhibition of stomata opening by abscisic acid [22, 23]. U73122 was used to study PLC in the light-induced stomata opening .The stomata guard cells after the inhibitor treatment speed up the opening caused by circadian rhythm. The question whether the PLC regulation of the response to the light influence occurs in the same way as the abscisic acid signalling, still remains open [74].

Thus, despite the recent significant achievements in the study on the PLC kinetics, gene expression and participation of this enzyme in the cell signal cascades, the mechanisms of realisation of PLC-mediated signalling pathway require further intensive many-sided research.

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Молекулярные основы реализации сигнального пути фосфатидилинозитол-специфической фосфолипазы С в клетках растений

Резюме

Сигналы из окружающей среды могут восприниматься и усиливаться в клетках с помощью сигнальных каскадов. У растений фосфатидилинозитол-специфическая фосфолипаза С (ФЛС) выполняет важную роль в клеточном ответе на внешние стимулы. Субстрат и продукты ФЛС регулируют множество процессов в клетках растений. В настоящем обзоре мы сосредоточили внимание на молекулярных основах реализации сигнального пути фосфатидилинозитол-специфической ФЛС. Анализ данных поможет расширить представление о механизмах, лежащих в основе способности растений реагировать на разнообразные абиотические и биотические стрессы.

Ключевые слова: фосфатидилинозитол-специфическая фосфолипаза С, трансдукция сигнала.

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Молекулярні основи реалізації сигнального шляху фосфатидилінозитол-специфічної фосфоліпази С у клітинах рослин

Резюме

Сигнали довкілля можуть сприйматися та посилюватися в клітинах завдяки сигнальним каскадам. У рослин фосфатидилінозитол-специфічна фосфоліпаза С (ФЛС) виконує важливу роль у клітинній відповіді на зовнішні стимули. Субстрат та продукти цього ферменту регулюють численні процеси в клітинах рослин. В огляді зосереджено увагу на молекулярних основах реалізації сигнального шляху фосфатидилінозитол-специфічної ФЛС. Аналіз даних може доповнити уявлення про механізми, що лежать в основі здатності рослин реагувати на різноманітні абіотичні та біотичні стреси.

Ключові слова: фосфатидилінозитол-специфічна фосфоліпаза С, трансдукція сигналу.

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