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REVIEW

Plasma Membrane-Bound Mechanisms of Signal Transduction in the Control of Plant Dormancy and Resistance

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Abstract—Data on membrane-bound biochemical mechanisms of control of plant dormancy and resistance to phytopathogens are discussed. Phytohormones are involved in the control of plant dormancy by the modification of activity of membrane-bound enzymes and ion channels. Similar constituents of the plasma membrane are influenced by fungal extracellular metabolites. Proposed interconnections between plasmalemma-bound signaling mechanisms responsible for plant resistance to infection and dormancy regulation are illustrated by a scheme.

Key words: phytohormones, dormancy, growth regulation, resistance, elicitors

The growth activity of perennial plants changes rhythmically during their life history: the period of active growth is followed by dormancy, when plant growth and development are arrested. A modification of plasma membrane function is involved in the break of dormancy [1]. Phytohormones play an important role in the control of dormancy by influencing the activity of membrane-bound enzymes [1, 2].

The break of dormancy can also be induced by invasion of pathogens. At the same time, the resistance to the infection varies depending on the physiological state of the plant [3, 4]. Extracellular metabolites of pathogens, like phytohormones, affect plasma membrane function in plant cells [5]. Thus, both phytohormones and fungal metabolites are signal substances inducing changes in plant cell metabolism.

According to current concepts, signal transduction from plasma membrane to genetic apparatus is realized via systems of adenylate cyclase, MAP-kinase, phosphatidic acids, phosphatidylinositol, lipoxygenase, superoxide synthase, and NO-synthase, as well through receptors possessing histidine kinase activity. The key enzymes of the systems (adenylate cyclase, kinase of kinase of MAP, phospholipases D, C, and A_2, NADPH-oxidase, and NO-synthase, respectively) are plasmalemma-bound enzymes that are activated by binding of signal substances with membrane-bound receptors. Further signal transduction is realized through a number of reactions that are specific to each signaling system, resulting in the alteration of activity of various protein kinases and protein phosphatases. These enzymes modify the factors of transcription regulation, whose interaction with gene promotor sites leads either to inhibition or stimulation of RNA and protein synthesis necessary to respond to incoming signal [6, 7].

The mechanisms of perception and transduction of phytohormone signal are found both in plasma membrane and cytoplasm [7]. However, the binding of phytohormone to cytoplasmic receptor depends on phytohormone concentration in cytoplasm, that is, on the rates of phytohormone inflow into the cell and output from the cell. The rate of phytohormone transfer across the plasma membrane is governed by the function of plasma-membrane-bound carrier proteins, H^+-ATPase, and ion channels [8, 9], whose activities can be modified both in the course of sprouting and by the action of metabolites of pathogens. Thus, the initial mechanisms of interplay between plant dormancy and resistance to pathogen may be assumed to function in the plasma membrane.

The mechanisms of plant dormancy regulation are now actively studied [1, 10]. The mode of hormonal signal perception and transduction in plants also are a major preoccupation of investigators [11-14]. A number of reviews are concerned with the molecular mechanisms of plant resistance to pathogens [6, 15, 16]. However, the

Abbreviations: ABA) abscisic acid; GA) gibberellic acid; GTP(γ)S) guanosine-5′-O-(3-thiotriphosphate); GDP(β)S) guanosine-5′-O-(2-thiodiphosphate); IAA) indole-3-acetic acid; MAP) mitogen activated protein kinase; PKC) protein kinase C.

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initial biochemical mechanisms of interconnection between plant dormancy and resistance remain unknown.

To elucidate the interrelation between membrane-bound mechanisms involved in the control of plant dormancy and resistance to pathogens, we shall discuss in this paper the effects of phytohormones and metabolites of plant pathogens on plasma membrane function.

EFFECT OF PHYTOHORMONES ON PLASMA MEMBRANE FUNCTION IN THE CONTROL OF DORMANCY

Physiological properties of seed and tuber dormancy, like optimal conditions of sprouting, are species- and cultivar-specific. For example, freshly harvested potato tubers cannot sprout even under favorable conditions because they are in deep dormancy. The duration of deep dormancy depends on the potato cultivar [17]. On the contrary, the seeds of many plant species do not possess deep dormancy. The sprouting of these seeds relies on environmental conditions (temperature, humidity, illumination) [10]. Despite the diversity of dormancy types, a great amount of data on the involvement of modification of plasmalemma functions in the sprouting of seeds and tubers of various plants has now been gathered. The modifications are induced both by the decrease in the content of growth-inhibiting phytohormones and by the increase in the concentration of growth stimulating ones in the cells of dormant organs. Peculiarities of plasmalemma activity changes vary with plant species, organ, and tissue; a common feature is a functional change in the plasmalemma at the end of dormancy [1, 2].

Potato tuber dormancy is under the control of ABA, GA, IAA, and cytokinins. It was found that GA, stimulating tuber sprouting, and ABA, inhibiting the sprouting, bind to some proteins of the plasma membrane of tuber parenchyma cells. The values of both binding constants and the numbers of binding sites change at the end of dormancy. The binding constant of protein to GA increased, whereas that to ABA decreased at the end of dormancy. After the end of dormancy, the number of GA binding sites in plasma membrane vesicles was lower, and that of ABA was higher than in samples obtained from dormant tubers [18]. The binding of phytohormones to the membrane resulted in changes in structure of some membrane proteins [1]. The value and specificity of the effects of GA and varied depending on tuber physiological state, suggesting that in dormancy and after dormancy the release of phytohormones influence different proteins of the plasmalemma [19, 20]. Further study revealed that the binding of phytohormones to plasma membrane proteins led to some changes in the properties of local regions on the surface of plasma membrane vesicles. However, the phytohormones neither affect the inner hydrophobic region of the membrane nor induce any global perturbations of the membrane structure [21].

Obviously, the effects of phytohormones on the properties of membrane proteins resulted in the changes of activity of membrane-bound enzymes: GA and IAA stimulated to different extent the activity of plasma membrane H^+-ATPase, whereas cytokinin and ABA inhibited this activity [22]. The study of ATP-dependent accumulation of Ca^{2+} in the inner space of plasma membrane vesicles obtained from potato tuber parenchyma showed that GA and ABA inhibited the activity of Ca^{2+}-ATPase to various extents, whereas kinetin stimulated the enzyme activity. At the same time, IAA stimulates the efflux of Ca^{2+} from the vesicles [23]. According to the modern view, Ca^{2+} mediates the transfer of external stimuli into plant cells, in part, by the regulation of PKC activity. The end of dormancy was accompanied by PKC activation by enzyme translocation from cytosol to plasma membrane [24]. Short-term treatment of potato parenchyma with GA led to decrease in PKC activity in cytosol and increase in the activity of membrane-bound PKC. GA did not influence the activity of other protein kinases that were also found in potato parenchyma cells. Apparently, inhibition of Ca^{2+}-ATPase by GA results in increase in Ca^{2+} content and the activation of PKC by its redistribution from cytosol to plasma membrane. PKC is involved in the phosphatidylinositol system of signal transduction. Recently, another key enzyme of this system, phosphoinositide-specific phospholipase C, was found in potato tubers [25]. These data suggest the function of the phosphatidylinositol system of hormonal signal transduction in potato tuber cells; the system can be activated by tuber sprouting under the influence of GA.

In the aleurone cells of germinating seeds of cereals, the synthesis and secretion into endosperm of α-amylase and other enzymes that hydrolyze storage nutriments necessary for growth of seedlings occur. GA along with stimulation of seed germination induces the synthesis and secretion of hydrolase. ABA inhibits these processes. The germination of barley seeds is accompanied by activation of outward rectifying K^+ channels in aleurone cells [26]. GA stimulated efflux of K^+ and Cl^- and Ca^{2+} inflow in the cytosol of aleurone cells [27]. As a result of GA treatment, phospholipase activation and modification of plasmalemma lipid layer, i.e., decrease in phosphatidylcholine and increase in inositol phospholipid contents, also were found in aleurone cells [28, 29]. The earliest known response to GA is the stimulation of phosphatidylinositol synthesis, observed 30 sec after the phytohormone treatment [30].

The level of membrane protein phosphorylation plays an important role in GA signal transduction: GA-induced increase in cytosolic Ca^{2+} content, as well K^+ and Cl^- efflux, and α-amylase gene expression were blocked by the treatment of aleurone cells with the protein phosphatase inhibitor okadaic acid [31].

ABA causes temporary hyperpolarization of aleurone cell membrane potential and an increase of intracellular pH. The H^+-ATPase inhibitors eliminated the effect of ABA on membrane potential and inhibited the ABA-induced expression of *RAB* genes (genes responsive to ABA). The K^+ channel blocker $BaCl_2$ prolonged the effect of ABA on membrane potential [32, 33]. The authors concluded that the ABA-induced activation of both H^+-ATPase and inwardly conducting K^+ channels are important factors involved in the regulation of hydrolase synthesis and *RAB* gene expression. The decrease in the intracellular Ca^{2+} content caused by the ABA-induced activation of the plasma membrane Ca^{2+}-ATPase inhibited both the expression of the α-amylase gene and the secretion of the enzyme [34-36].

Phospholipase D is also among the ABA-activated enzymes in aleurone cells of barley [37]. Increase in the enzyme activity was accompanied by increase in the intracellular concentration of phosphatidic acid. The phospholipase D inhibitor 0.1% butanol inhibited the ABA-induced processes. The addition of phosphatidic acid restored the cell sensitivity to ABA. Phosphatidic acid added to the incubation medium in the absence of ABA mimicked the phytohormone effect on the intracellular Ca^{2+} content and α-amylase production and induced synthesis of the same proteins as ABA did.

It has been shown that in animal cells phosphatidic acid or its metabolite lysophosphatidic acid, a product of the phosphatidylcholine hydrolysis by phospholipase D, is a secondary messenger in the regulation of activities of protein kinases, G proteins, phosphatidylinositol kinases, adenylate cyclases, and other signal molecules [38]. It is possible that phosphatidic acid plays a similar role in plant cells.

At the end of dormancy, the function of the plasma membrane of germ axis cells is also under hormonal control. The germination of radish seeds was accompanied by hyperpolarization of the germ axis cell membrane potential, as well as augmented H^+ release and K^+ influx. GA stimulated, and ABA inhibited, these processes [39]. At the end of dormancy of rice seeds, the activation of serine-threonine-dependent protein kinase in embryonic cells was noted. The ABA-induced inhibition of rice seed germination led to the inhibition of the enzyme [40]. GA stimulated both germination and the enzyme activity [41].

Direct evidence of GA and ABA binding to the plasma membrane of aleurone cells is lacking. GA and ABA affected α-amylase synthesis only when applied externally. A microinjection of GA and ABA into the protoplasts of aleurone cells did not influence the synthesis and secretion of the hydrolase [42] suggesting the necessity of the interaction of the hormone with plasma membrane constituents. It should be noted that GA and ABA influenced the synthesis of the hydrolases only when the pH value of incubation medium was low. Under this condition, weak organic acids GA and ABA are in their non-dissociated state and can penetrate the cell with their concentration gradient. It is not inconceivable that precisely the binding of these non-dissociated molecules of GA and ABA to an appropriate receptor in cytoplasm or on inner surface of plasma membrane induce the chain of reaction resulting in the synthesis and secretion of the enzyme. Participation of G proteins and cyclic GMP in the GA-induced α-amylase synthesis [43, 44] may be an indirect indication of the existence of GA binding sites on the plasma membrane.

Thus, the change in activity of ion channels and membrane-bound enzymes—H^+-ATPase, Ca^{2+}-ATPase, phospholipases, protein kinases, and protein phosphatases—is clearly involved in dormancy regulation by phytohormones as the initial step of hormonal signal transduction. It seems to be very likely that each of these enzymes is a link of the chain of specific reactions controlling various metabolic processes. For example, phospholipases C and D are the key enzymes of phosphatidylinositol and phosphatidic acid systems, respectively. Ca^{2+}-ATPase and Ca^{2+} channels are involved in the regulation of cytosolic free Ca^{2+} concentration, which is a second messenger in all studied signal-transducing systems. The activity of a number of protein kinases and protein phosphatases depends on the free Ca^{2+} concentration. The plasma membrane H^+-ATPase is involved in the regulation of the value of cytoplasmic pH and membrane potential, which controls the transfer of low molecular weight metabolites into cells. Additionally, the activity of plasmalemma K^+, Cl^-, and Ca^{2+} channels depends on H^+-ATPase function. In turn, modification of K^+ and Cl^- channels activity leads to change in both Ca^{2+} channel activities and cell turgor. Moreover, the inflow of the phytohormone into the cell depends on the value of membrane potential caused by H^+-ATPase function [8, 9]. The possibility of one phytohormone influence the binding of another to its cytoplasmic receptor by modification of H^+-ATPase activity cannot be ruled out.

INFLUENCE OF PATHOGENS ON HOST PLASMA MEMBRANE FUNCTIONS

The invasion of a pathogen into plant tissues results in either the development of disease or resistance achieved by several biochemical mechanisms. More investigated is the resistance caused by hypersensitivity, which appears as rapid death of invaded plant cells as the result of action of several metabolites called elicitors [45]. Depending on fungal type and plant species, hypersensitivity either occurs (incompatible combination, resistance) or does not develops (compatible combination, susceptibility). The components of fungal and plant cell walls and metabolites excreted by the fungus, such as oligosaccharides, glycopeptides, proteins, and fatty acids are known as elicitors [5].

In the initial stages of infection, the cell wall of fungal hypha is in close contact with the plant cell plasma membrane. Therefore, the phytotoxic hyphal wall components and extracellular metabolites can influence the structure and function of the plant plasma membrane [46, 47]. Several signal transduction pathways lead to various responses in different plants. Even in the same plant, different elicitors activate different signal transduction chains, although the induced defense responses may be, at least in part, similar [5]. The following discussion will be focused on different possible signal transduction mechanisms rather than on individual elicitor signaling pathways.

The modification of ion transport and plasma membrane potential is the earliest response of a plant cell to elicitor treatment. The stimulation of K^+ and Cl^- efflux and Ca^{2+} influx, as well the change of incubation medium pH value were observed in the first minutes after elicitor addition, this correlating with the activation of a defense response [48, 49]. The plasma membrane potential value and ion cannel activity are related to function of the plasma membrane H^+-ATPase. Depending on the nature of the elicitor and the plant species, elicitors stimulated or inhibited enzyme activity [50-52].

Plasmalemma anion channels play an important role in elicitor-induced membrane depolarization. The anion channel activation can induce efflux of Cl^- and inflow of Ca^{2+} into the cytoplasm. Anion channel blockers inhibited both elicitor-induced defense response and Ca^{2+} inflow into cytosol [49, 53]. In the cells of soy, parsley, and tobacco the omission of Ca^{2+} from the incubation medium inhibited the activity of the defense genes and phytoalexin accumulation [54-56]. Increase of cytoplasmic Ca^{2+} concentration from 0.1 to 2 μM was found 1-2 min after the addition of β-glucan elicitor in the cells of soy suspension culture [57]. Elicitor prepared from *Phytophthora infestans* hypha cell walls stimulated Ca^{2+} efflux from potato tuber cells followed by oxidative burst. The Ca^{2+} channel blocker verapamil eliminated the elicitor effects [58]. The treatment of tomato leaf protoplasts with race-specific peptide elicitor from *Cladosporium fulvum* activated plasma membrane Ca^{2+} channels and inhibited the activity of Ca^{2+}-ATPase [59, 60]. In parsley leaf protoplasts a Ca^{2+} channel reversibly activated by oligopeptide elicitor from *Phytophthora sojae* cell walls has been found [61]. The same building blocks of the elicitor were necessary for the induction of defense genes, phytoalexin synthesis, and Ca^{2+} channel activation.

The activation of Ca^{2+} channels resulting the stimulation of Ca^{2+} inflow into the cell is essential to regulation of the level of phosphorylation of plant proteins [59, 62-65]. Protein kinase inhibitors eliminated an elicitor-induced ion efflux, protein phosphorylation, and plant defense response. The inhibition of protein phosphatase activity stimulated the plant defense response in the absence of elicitors [66]. Obviously, the elicitor effect involves the activation of phosphorylation.

One of the specific plant responses to infection or elicitor treatment is so-called oxidative burst, the generation of active oxygen species (H_2O_2, superoxide radicals) [67]. In the case of incompatible combination, two-phase kinetics of H_2O_2 accumulation was observed, whereas in compatible combination active oxygen species either are not produced or one small peak of oxidants was found [68, 69]. The release of active oxygen species in the oxidative burst affects the attacking pathogen and the host cell at the infection site, inducing localized hypersensitivity. The oxidative burst led to the cross-linking of cell wall proteins, rendering the plant cell wall more resistant to attack by pathogen enzymes [70]. The active oxygen species are toxic for pathogen and, additionally, may serve as second messengers for the activation of genes expressing protective proteins. In some of host–pathogen systems the generation of active oxygen species closely correlates with the accumulation of phytoalexins [71], compounds that are produced in response to infection or elicitor treatment and induce the death of treated cells.

Data on the involvement of plasma membrane NADPH- and NADH-oxidases in the oxidative burst have accumulated. In phagocyte cells, the oxidase electron-transfer chain of the plasma membrane includes a heterodimeric protein, cytochrome b_{558}, which consist of subunits gp91-*phox* and p22-*phox*. The enzyme is activated by association with cytoplasmic proteins p47-*phox*, p67-*phox* and a monomeric G protein (rac2). The interaction between the phosphorylation-activated SH_3 domains of p47-*phox* or p67-*phox* proteins and proline-enriched sequence of p22-*phox* is necessary for the self-assemble [72]. In soy, parsley, and arabidopsis cells, elicitor-induced oxidative burst was eliminated by NADPH-oxidase inhibitor from mammalian cells [71]. Antibody to p47-*phox* and p67-*phox* from human neutrophils interacted with proteins of the same molecular mass from soy, cotton, arabidopsis, and tomato plants [73]. The treatment of tomato leaf cells with race-specific elicitor from *C. fulvum* induced the translocation of p47-*phox*, p67-*phox*, and rac2-like proteins from the cytoplasm to the plasma membrane [74]. This process depends on the level of the phosphorylation of respective proteins, but in plant cells, unlike animal cells, PKC-activators and inhibitors had no effect on enzyme self-assembly and activity. However, oxidase activity and self-assembly were inhibited by the chelator ethylene glycol-bis-(amino-ethyl ether) N,N,N′,N′-tetraacetic acid and by calmodulin antagonist [74] suggesting the involvement of Ca^{2+}/calmodulin-dependent protein kinase in the induction of oxidative burst. The inhibitors of tyrosine and serine-threonine protein kinases eliminated the effect of elicitor in soy cell suspension culture. The ion-channel blocker anthracene-9-carboxylic acid inhibited oxidative burst induced by elicitor in similar

sojae cells, although ion-channel generator amphotrecin B simulated the effect of elicitor [75]. Oxidative burst induction is apparently related to the activation of NADPH-oxidase in the plant cell plasma membrane similarly to phagocyte plasma membrane. Mechanisms of such activation in plant cells and phagocytes have much in common.

Membrane-bound phospholipases are also involved in the production of active oxygen species. Phospholipases A, D, or C are activated depending on host plant species and elicitor nature [76]. In soy cell suspension culture, the induction of oxidative burst by octylgalacturonic acid is related to the activation of phospholipase C [77], whereas other elicitors (harpine, *Verticillium dahliae* extract) activated phospholipase A during oxidative burst induction. The phospholipase A inhibitor chlorpromazine-HCl inhibited oxidative burst induced by elicitors from *V. dahliae* but had no effect on the active oxygen species production induced by other elicitors [78]. The same plant response to elicitor can apparently be mediated by different mechanisms.

Rapid lipid oxidation caused by oxidative burst or by phospholipase A and lipoxygenase activation was seen in many plants treated by elicitors [79]. Unsaturated fatty acids released from membrane lipids by phospholipase A_2 can activate protein kinases or to be oxidized by lipoxygenases, thus being transformed to hydroperoxy-derivatives generating compounds toxic to microorganisms and also phytodienic and jasmonic acids [80]. These compounds can serve as second messengers in the induction of defense gene expression and specific pathogenesis-related protein synthesis. The function of membrane lipids and the derivatives of their oxidation in the cell response to infection have been detailed in several reviews [5, 6, 76].

Consequently, the effect of elicitors cause significant changes in the functions of plant cell plasma membrane required for signal transduction and induction of plant defense responses. Such responses are triggered by the binding of elicitors to plasma membrane proteins. Specific, reversible, and saturable binding of β-glucans to the plasma membrane was found in soy root cells and protoplasts obtained from soy cell suspension culture [81, 82]. One kind of binding sites for hepta-β-glucoside with K_d 1-3 nM and for β-glucan fraction from *P. sojae* with K_d 10-40 nM was determined in plasma membrane. These K_d values are in good agreement with the ligand concentration required for the expression of biological activity [81, 83]. Proteins (75, 100, and 150 kD) binding β-glucans from *P. sojae* were solubilized and isolated [84]. A direct correlation was found between biological activity of different glucosides and their ability to compete with ^{125}I-labeled elicitor for the binding sites [85]. Cyclic (1,3–1,6)-glucans from the bacterial symbiont *Bradyrhizobium japonicum* inhibited both the induction of defense response by *P. sojae* β-glucans and their binding to plasma membrane [86]. To date, the binding sites for β-glucan elicitors have been found only on the cell plasma membrane of Fabaceae. Taxonomically related plants are assumed to have similar mechanisms for microbial oligosaccharide recognition [87].

Similar kinds of binding sites with high affinity to elicitor-active chitin fragments N-acetyl-chitopentaose and N-acetyl-chitooctose were revealed in the plasma membrane of tomato and rice respectively [88, 89]. Deacetylated chitooligosaccharides did not compete with chitin fragments for binding sites and did not stimulate plant defense response [90, 91]. Proteins of 75 kD binding chitooligosaccharides were solubilized from rice cell plasma membrane [92].

Specific binding sites with high affinity to peptidoglucans and glycopeptides were found in wheat cell membrane [93]. Highly specific saturable and reversible ligand binding was revealed in tomato cells and microsomal fraction obtained from these cells. Mannose oligosaccharides obtained by enzymatic digestion of glycopeptide elicitor inhibited its biological activity and competed for binding sites with radiolabeled glycopeptide [94]. High affinity binding sites for the race-specific peptide elicitor AVR-9 from *C. fulvum* were revealed on tomato leaf cell plasma membrane [95]. A radiolabeled oligopeptide obtained from 45-kD glycoprotein elicitor from *P. sojae* bind to microsomes and protoplasts from parsley leaf cells in specific, saturable, and reversible manner. The same oligopeptide structures are required for the binding to membrane and the induction of defense response [56]. In specific cases, elicitor-binding proteins were found to be serine-threonine protein kinases and kinase activity was required for both interaction with peptide elicitor and the development of defense response [96-98].

Consideration of existing data suggest that in most cases elicitor binding to the cell membrane proteins of a host plant fits the criteria taken for ligand–receptor interaction: specificity, reversibility, high affinity to ligand, and saturability of binding sites were demonstrated. Quantitative correlation was found between affinity of ligand or its structural analogs to binding sites and the capacity to induce biological response. The involvement of G-proteins in the plant response to pathogen is additional evidence for receptor function of elicitor-binding proteins [99-102]. Induction of oxygen active species production by the soy elicitor octylgalacturonic acid in soy cell suspension culture is related to the activation of heterotrimeric G-proteins [99]. The treatment of tomato leaf cell suspension culture by race-specific elicitor from *C. fulvum* resulted in increase in ferricyanide reduction and the activation of NADH-oxidase and NADH-dependent cytochrome *c* reductase in plasma membrane as well as inhibition of ascorbate oxidase [59]. The non-hydrolyzable GTP analog, GTP(γ)S, maintaining heterotrimeric G-protein in the active form bound to GTP influenced ferricyanide reduction and the activity of

NADH-oxidase and ascorbate oxidase similarly to the elicitor. Stimulation by elicitor was not evident in the presence of GDP(β)S, supporting G-protein in the inactive form bound to GDP. Activator of G-protein promoting nucleotide metabolism similarly to the natural receptor, mastoparan, stimulated ferricyanide reduction and NADH-oxidase activity and inhibited ascorbate oxidase similarly to elicitor and GTP(γ)S. However, the activity of NADH-dependent cytochrome *c* reductase was not altered in the presence of guanyl nucleotide analogs [63]. Apparently several pathways of signal transduction can operate in tomato cells for the activation of one of the same defense responses, one of them mediated by G-protein. The activation of Ca^{2+} channels observed in tomato leaf cell protoplasts by the same elicitor treatment was prevented by GDP(β)S, while GTP(γ)S and mastoparan simulated the effect of the elicitor. In tomato cell suspension culture, elicitor-induced dephosphorylation of plasma membrane H^+-ATPase leading to increased enzyme activity was also related to the activation of heterotrimeric G-proteins [100]. In spruce root cells, the efflux of Cl^- and K^+ and alkalinization of the external medium were the first responses to the treatment with the elicitor N-acetylglucosamine [101]. Mastoparan induced similar reactions while the mastoparan analog Mas-17 that failed to stimulate G-protein had no influence on ion efflux and change of extracellular pH value. Both oligosaccharide elicitors and mastoparan induced oxidative burst in parsley cell suspension culture [102].

Thus, a correlation between heterotrimeric G-protein activity and cell response to the treatment with elicitor undoubtedly exists. Plant response is apparently realized by ligand–receptor interaction that activates G-proteins. At the same time, the reception of elicitor alone is apparently not the only condition for the induction of defensive reactions. Cell plasma membrane of susceptible tomato cultivars was found to have binding sites with high affinity for race-specific peptide elicitor from *C. fulvum* [95]. The same is true for other solanaceous plants that do not produce visible necroses when treated with elicitor. The development of hypersensitivity is likely blocked because the absence of required constituents of the signal transduction chain or the inhibition of appropriate reactions.

It can be concluded that plant resistance to phytopathogenic fungi can be related to the interaction of pathogen metabolites with a receptor protein on the plant cell membrane. This interaction leads to multiple changes in the functional activity of the cell plasma membrane. At this time, fungal metabolites and phytohormones controlling dormancy affect the activity of the same membrane enzymes. The few available data on the biochemical mechanisms of the relation between the action of phytohormone and fungal metabolite on plant plasma membrane will be reviewed below.

INTERFERENCE OF PHYTOHORMONE AND PATHOGEN METABOLITE EFFECTS ON PLANT CELL PLASMA MEMBRANES

The changes caused by fungus in plant tissues are known to depend not only on the fungal species but also on the developmental stage of the plant [4]. Resistance to infection by some fungi is higher in dormant than in sprouting potato tubers [103]. The infection of dormant tubers leads to their sprouting.

The influence of phytohormones on plant–fungus interaction is well established [104, 105]. The phytohormone effect also depends on the physiological state of the plant. Pretreatment with ABA stimulated phytoalexin accumulation and hypersensitivity development in dormant potato tuber tissues infected by an incompatible *P. infestans* race, while after the end of dormancy ABA inhibited these reactions [106].

Arachidonic acid, an elicitor from *P. infestans*, induced hypersensitivity in the potato tuber tissues after the end of dormancy and inhibited the activity of cation-stimulated ATPase in a preparation of potato tuber cell plasma membrane. Pretreatment with ABA blocked both the arachidonic acid induced hypersensitivity and the inhibiting effect of the elicitor on the activity of the plasma membrane ATPase [107]. Additionally, metabolites of *P. infestans* were found to prevent the reception of ABA [108]. These data demonstrate the important role that ABA plays in plant–fungus interaction. It can be suggested that disturbance in ABA reception is one of the factors in the induction of hypersensitivity.

Only plasma membrane preparations from potato tubers were employed in experiments on the interference of extracellular fungal metabolites and phytohormones on binding with plasma membrane. The results give some idea about the functions of the sites of plasma membrane binding of fungal metabolites [109].

Plasma membrane preparation from potato tubers was shown to bind extracellular metabolites of *P. infestans* [110] that contained both proteins and glycoproteins greater than 1 kD with 1 : 1 carbohydrate/protein ratio [111].

The influence of fungal metabolites on the binding of phytohormones to plasma membrane depended on both physiological state of the potato tubers and the fungal race. Fungal metabolites did not influence the binding of GA and ABA to the plasma membrane from dormant tubers [112]. Some assumed influences of fungal metabolites on receptor in plasma membrane obtained from potato tubers after the end of dormancy are represented schematically in Fig. 1. ABA did not bind to plasma membrane from non-dormant tubers in the presence of metabolites from incompatible fungus race (race to which the used potato cultivar is resistant) perhaps because of the blocking of the receptor (Fig. 1b). It can be supposed that the result of such effect of metabolites will be the

stimulation of growth processes inhibited by ABA or the change in the plant cell caused by the disruption of balance between the phytohormones acting on the cell. It should be remembered that the binding constant of plasma membrane sites to ABA is lowered at the end of potato tuber dormancy [18]. The metabolites of compatible race (that can infect the potato cultivar) did not prevent the binding of ABA to plasma membrane and increased the binding constant. Apparently, fungal metabolites not only compete with ABA for the binding sites (in incompatible interaction) but also change the ability of plasma membrane to bind ABA (in compatible interaction).

A result of treatment with metabolites from the compatible race is that the number of binding sites of IAA to plasma membrane significantly increased, perhaps as a result of removal from the surface of the plasma membrane molecules that shielded some receptors (Fig. 1c). The removal of some component from plasma membrane surface was shown to occur on the action of the fungal metabolites [110]. The metabolites from the incompatible race did not alter the binding parameters of GA, while the number of binding sites decreased in the presence of a compatible race. The possibility exists that the effect of compatible fungal metabolites on GA binding is similar to the effect of incompatible metabolites on ABA binding (Fig. 1b). As mentioned above, the number of the sites of GA binding to a plasma membrane preparation decreased at the end of tuber dormancy [18].

Stimulation of potato tuber sprouting by infection with *P. infestans* and simultaneous decrease in the number of binding sites for certain phytohormones by the action of fungal metabolites [112] suggest the possibility of imitation of phytohormone action by fungal metabolites in the case of complementarity of the shape of the metabolite molecule to the site of phytohormone binding (Fig. 1d).

Evidently, not only the fungal metabolites penetrating in plant tissues influence the binding of phytohormones to plasma membrane, but phytohormones existing in the plant influence the interaction of fungal metabolites with the host cell plasma membrane. Investigation of the influence of phytohormones on the interaction of fungal metabolites with the plasma membrane from sprouting potato tubers showed that ABA did not alter the binding of incompatible metabolites, while IAA completely inhibited it. GA did not influence the binding of fungal metabolites to plasma membrane vesicles.

Thus, fungal metabolites can both bind to the host plant plasma membrane and modify its properties leading to a change in phytohormone binding parameters and modification of processes controlled by respective phytohormones.

Pathogen invasion, like sprouting, induces a chain of reactions leading to the synthesis of a set of specific proteins. Change in functional activity of plasma membrane induced by phytohormones and fungal metabolites play a role of trigger of these reactions. Interference of phytohormones and fungal metabolites at the reception on the plasma membrane can be the first level of the interaction between these signal substances (Fig. 1).

Biochemical processes triggered on the plasma membrane by sprouting or the action of fungal metabolites have much in common (Fig. 2). Ion channels, H^+-ATPase, phosphorylation cascade, phospholipases, and Ca^{2+}-ATPase all are involved in the chain of signal transduction of both phytohormones and fungal metabolites. It is highly probable that these membrane components serve as members of signal transduction chain for both phytohormones and fungal metabolites. In such a case, the effect of phytohormones and fungal metabolites on these membrane components is important for the interplay between dormancy and resistance to disease.

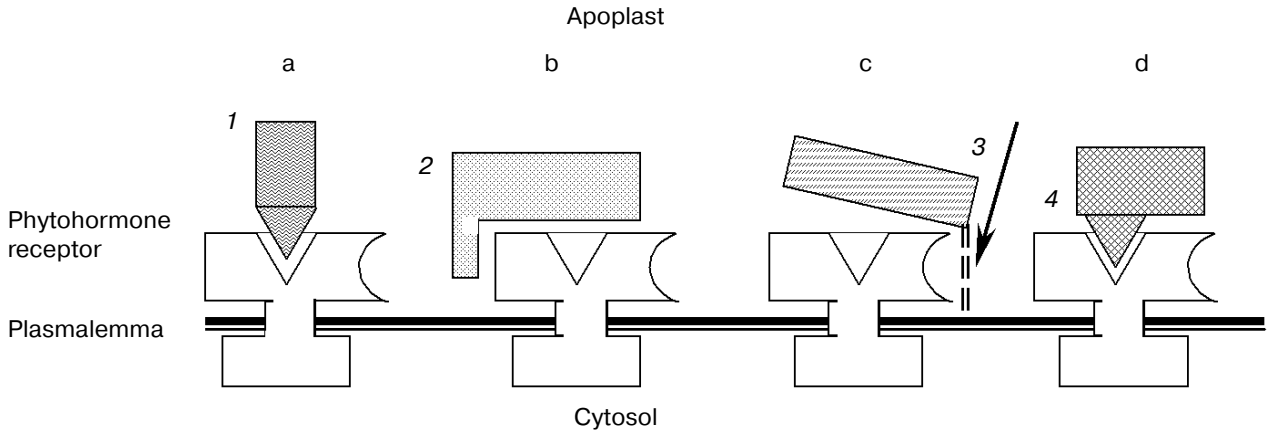

Fig. 1. Diagram of hypothetical effect of extracellular fungal metabolites on plant receptors for phytohormone: a) regular interaction of phytohormone with a receptor; b) the receptor is blocked; c) elimination of shielded molecule; d) simulation of phytohormone effect by fungal metabolite; *1*) phytohormone; *2*) fungal metabolite that blocks phytohormone receptor; *3*) action of fungal enzyme (shown by arrow); *4*) fungal metabolite that is bound to receptor.

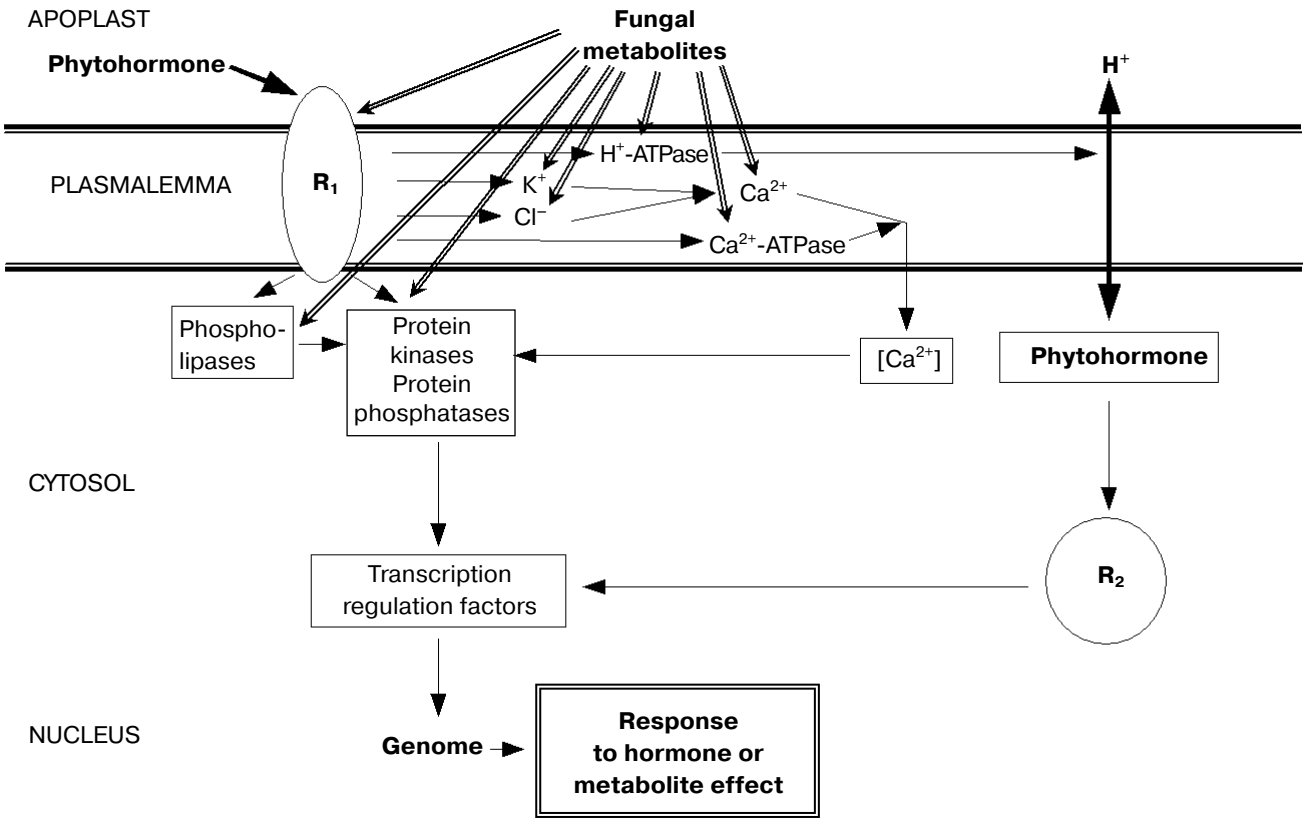

Fig. 2. Interaction of extracellular fungal metabolites and phytohormones in the control of dormancy and disease resistance. R_1 and R_2) receptors of phytohormones located on plasma membrane or in cytoplasm, respectively; K^+) K^+-channels; Cl^-) anion channels; Ca^{2+}) Ca^{2+}-channels; [Ca^{2+}]) concentration of free Ca^{2+} in cytosol. Double arrows show the action of fungal metabolites on the functioning of plasma membrane components controlled by phytohormones.

Phytohormone, either stimulator or inhibitor of sprouting, interacts with receptor in the plasma membrane and induces a chain of reactions promoting either retention of dormancy or beginning of sprouting depending on the nature of the phytohormone. During pathogenic infection, extracellular fungal metabolites influence the activity of members of the hormonal signal transduction chain leading to modification of physiological response to phytohormone. The end of dormancy is accompanied by a change in ratio between growth stimulators and inhibitors in the apoplast. This change alters the activity of members of the hormonal signal transduction chain and their sensitivity to the influence of fungal extracellular metabolites.

According to current concepts, the key members of adenylate cyclase, mitogen activated protein kinase, phosphatidic acids, lipoxygenase, superoxide synthase, and NO-synthase signal systems mediating the influence of external stimuli are located on the plant cell plasma membrane. The influence of external regulating stimulus on the activity of the members of one of these systems can lead to the changes in the activity of other systems and to modification of the response to the initial signal [6]. The interaction of signal systems can serve as one of the means of interaction between the effects of phytohormones and fungal metabolites. For example, phytohormone-induced modulation of the activity of Ca^{2+}-ATPase and Ca^{2+}-channels in the plasma membrane changes the content of cytosolic free Ca^{2+} that influence on the activity of plasma membrane enzymes (adenylate cyclase, phospholipases, NADPH-oxidase) that are involved in elicitor signal transduction via corresponding signaling systems (adenylate cyclase, phosphatidic acid, lipoxygenase, and superoxide synthase). In turn, the action of elicitors on the H^+-ATPase activity results in the modification of transmembrane electrochemical H^+ gradient governing the influx of phytohormones into the cell and their binding to cytoplasmic receptor. Elicitor-induced modification of the activity of plasma membrane K^+- and Cl^--channels causes change in transmembrane potential value that is an important factor controlling the activity of a set of enzymes involved in hormonal signal transduction.

A deficiency of information about the influence of fungal metabolites on the functional activity of plasma membrane components in the cells of dormant and

sprouting plant organs prevents precise determination of the constituents in the plasma membrane that are the points of interaction between fungal metabolites and phytohormones in the control of dormancy. The mechanisms of such interaction obviously depend on the set and nature of metabolites produced by the pathogen and on the competence of the plant tissue infected by the pathogen.

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