Molecular communications during plant–pathogen interactions

Ashalatha S. Nair

Department of Biology, Kyungpook National University, Taegu, 702 701, South Korea

Plant–microorganism interaction accounts for a three-step phenomenon leading from signal generation to cell responses, via transduction pathways. Signal transduction pathways are not clearly defined in plant cells. This paper is a short review integrating general features of plant–pathogen interaction, with special emphasis on different signal transduction pathways.

THE interaction between plant and pathogen involves an extensive and continuing exchange of information, evoking defence responses in plants and pathogenic traits in microorganisms. As defence responses, plant cells produce and accumulate phytoalexins, hydroxyproline-rich glycoproteins or enhance the activity of certain hydrolytic enzymes. Among these, phytoalexins appear to have a critical role against pathogen attack. The biochemical pathways for phytoalexin synthesis have already been defined[1] and the signalling molecules inducing their production reviewed by Darvill and Albersheim[2]. Compounds like rhaphiolepsin, scoparone and camalexin have been identified as phytoalexins[3–5] and new methods using HPLC and microspectrometry for isolating them from plant cells have been reported recently[6, 7]. Reviews on various biochemical, physiological and molecular aspects of induced defence response in plant cells have been published[8, 9]. Here we discuss various molecules involved in plant–pathogen interactions and review specifically the recent developments in signal tranduction pathways.

Signal molecules

In the past twenty years, several signal molecules have been isolated from both plant and microbial cell wall fragments. They are grouped into three classes according to their origin and function.

Elicitors

Glycans, peptides, glycoproteins or polysaturated fatty acids isolated from microbial cell wall fragments induce defence responses in plant cells. Extensive studies were carried out in elicitor molecules soon after the isolation of first biotic elicitor-monilicolin-A from the mycelia of *Monilinia fruticola*[10]. Recently, a polygalaturonase isolated from *Botrys cinerea* was found to exhibit eliciting activity in beans[11]. Chitosan was found to be a potent elicitor in oat leaves for the production of the phytoalexin avenamulin[12]. Detergents, heat and UV irradiation also produce similar responses in plant cells; hence, they are known as abiotic elicitors[2].

Inducers

Plant cell wall components or cell exudates such as pectic polymerases, cutin monomers and flavanoids trigger synthesis of several hydrolytic enzymes in the microbial cells. When microbial cells were grown in the presence of pectic polysaccharides, production of pectinases was increased. In the fungal spores of *Fusarium solani* f. sp. *pisi*, cutinase production was enhanced in the presence of cutin or its derivatives[13]. Flavanoid molecules from the plant cell induce *vir*-genes in *Agrobacterium*[14] and *nod*-genes in *Rhizobium*[15].

Suppressors

The polysaccharides or phenolics originating either from the microbes or from the plant cell wall fragments lower the induction of pathogenicity in pathogens and of defence responses in plant cells. Although most of the suppressors have not yet been characterized fully, they are structurally related to elicitors[16].

Signal transduction

During plant–pathogen interaction, it is presumed that elicitor molecules interact with the primary target sites(s) in plasma membrane. High-affinity binding sites of glucan elicitors have been identified in soybean membrane fractions[17]. The binding process was highly ligand-specific, was abolished by pronase treatment and stabilized in the presence of dithiothreitol. These results indicate that the binding sites might be a protein. Even though a putative receptor molecule has not yet been purified, evidence for specific binding sites is clearly established.

A rapid, partial membrane depolarization was observed in seconds when elicitor preparations from *Colletotrichum lagenarium* and *Phytophthora paracitica* var *nicotiana* were applied to their host roots[18]. The rapidity of this effect supports the hypothesis that elicitors act primarily on cell surface level. Also, this totally reversible effect indicates that elicitors do not damage plasma membrane. After this primary effect, how the signals are transducting to initiate defence genes is not well defined.

In suspension-cultured bean cells, elicitor treatment induces the synthesis of chitinase and other enzymes for the production of phytoalexins within 2–3 min, suggesting only a few intervening steps between elicitor binding to a receptor and specific transcriptional activation of defence genes[8]. The simplest model for the induction of plant defence responses assumes that the elicitor binds to a specific receptor, probably located in plasma membrane, and the signals are transducted through ethylene, through protein kinase enzymes or through polyunsaturated-fatty-acid-derived compounds, leading to changes in the transcriptional activity of defence genes.

The significance of ethylene as a signal to activate plant defence gene has been known for a long time[19, 20]. Elicitors of ethylene from *Colletotrichum lagenarium* trigger chitinase activity in melon plants[21]. A fungal elicitor isolated from *Trichoderma viridae* elicits enhanced ethylene synthesis and tissue necrosis in tobacco plants[22]. Another proteinaceous fungal elicitor isolated from *Phytophthora cryptogen* initiated ethylene synthesis and accumulation of proteinase inhibitors in tobacco plants[23]. An increase in ethylene synthesis was observed within 15–30 min after elicitor treatment. This is a transient reaction, occurring through the rapid stimulation of ACC-synthase and other membrane-bound enzymes[24]. The rapid synthesis of ethylene after elicitor treatment indicates that this *plant hormone could be a signal carrier molecule.* Furthermore, role of ethylene in transcriptional regulation of defence genes like PAL, 4-CL, HGRP and CHS has already been demonstrated in carrot[25]. Ethylene is also involved in the modulation of Win2 (wound-induced) gene expression[26]. These results indicate a signal transduction pathway through ethylene leading to the transcriptional activation of defence genes in plant cells.

In tomato and potato, the chemicals that signal the expression of proteinase inhibitor genes, induce the phosphorylation of plasma membrane proteins[27]. These phosphorylations are often due to the modulations of protein kinase activity, the kinase often being a part of, or associated with, the receptor itself, eventually regulating biochemical reactions leading to the regulation of other metabolic processes. A rapid, transient and sequential phosphorylation of proteins has been shown by electrophoretic analysis in Parsely cell cultures treated with fungal elicitors[28]. A microsomal phosphoprotein pp45-kDa appears almost immediately upon elicitor treatment and this could be involved in signal recognition/transduction process originating from plasma membrane. A nuclear protein npp26-kDa also becomes phosphorylated early in the sequence of events and could play a role at the end of signal transduction chain leading from plasma membrane to gene activation in the nucleus. There is no conclusive evidence for direct relationship between protein phosphorylation and activation of defence genes. However, both events are triggered by proteinaceous components of elicitors and apparent Ca^+ dependence of both processes supports the idea of a signal transduction pathway involving Ca^+-dependent protein kinase enzymes and signal amplification through a cascade mechanism.

In search of a signal chain between the elicitor–receptor complex and the gene activation process, Gundlach *et al.*[29] identified rapid and transient accumulation of jasmonic acid and its methyl esters in suspension cultures of *Rauvolfia canescens* and *Eschscholtzia californica* after treatment with yeast elicitors. These compounds act as natural hormone regulators, inducing the synthesis of defensive proteinase inhibitor proteins, and are transported as volatile chemicals like ethylene[30]. Discovery of jasmonic acid as a signal transporter supports a transduction pathway based on polyunsaturated fatty acids and its derivatives. An elicitor–receptor complex activates a lipase, thereby releasing α-linolenic acid, which is then transformed by constitutive enzymes to jasmonic acid and methyl jasmonate. Methyl jasmonate induces the transcription of PAL gene, resulting in elevated levels of phenylalanine ammonia lyase, the first enzyme in the synthesis of lignin and other isoflavanoid phytoalexins.

Response to signal molecules

The ultimate response to microbial signals is concerned with the induction of defence genes. Defence genes such as PAL are generally encoded by a family of three genes. It has been shown that individual members of the gene family exhibit markedly different patterns of regulation with respect to organ-specificity during normal development as well as within a single organ in response to diverse environmental stimuli[31]. This selective expression of genes was governed by a complex set of unknown regulatory networks.

To investigate the early event in the activation of resistant mechanisms, the expression of a chimeric gene comprising the 5' flanking region of a defence gene encoding chalcone synthase (CHS) fused to a bacterial chloramphenicol acetyl transferase (CAT)

gene and the 3' flanking region of the nopaline synthase (NOS) gene was examined, followed by electroporation into soybean protoplasts[32]. Functional analysis of 5' deletions revealed that the promotor activity is determined by an elicitor-regulated activator located between the 'TATA' box and nucleotide position −173 and an upstream silensor between −173 and −326. These *cis*-acting elements function in the transduction of the elicitation signal to initiate the elaboration of an inducible defence response. From the studies revealing the structure of phenylalanine ammonia-lyase gene in Parsely, the PAL-1 promoter region demonstrated two nucleotide sequences, within the motifs CTCCAACAAACCCCTTCC and ATTCTCACCTACCA, involved in the response to both UV irradiation and elicitor application[33]. These motifs are conserved at similar positions in several elicitor or light-responsive genes and might play a general role as *cis*-acting elements.

A family of chitin-binding proteins with a conserved chitin-binding domain was reported from plants ranging from monocots to dicots. These proteins play an important role in plant defence system[34]. Several genes responsible for the production of chitin-binding proteins have been isolated and the proteins characterized[35]. Transgenic plants containing these genes may be helpful in understanding the missing factors during plant–pathogen interactions and subsequent cell responses.

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