Invited Trends Article

Elicitor Recognition and Signal Transduction in Plant Defense Gene Activation

Dierk Scheel

Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, D-5000 Köln 30, Bundesrepublik Deutschland

and

Jane E. Parker

Sainsbury Laboratory, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

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Plants defend themselves against pathogen attack by activating a whole set of defense responses, most of them relying on transcriptional activation of plant defense genes. The same responses are induced by treatment of plant cells with elicitors released from the pathogen or from the plant surface. Several plant/elicitor combinations have been used successfully as experimental systems to investigate the molecular basis of plant defense responses. Receptor-like structures on the plasma membrane of plant cells appear to bind the elicitors. Thereby, intracellular signal transduction chains are initiated which finally result in the activation of plant defense genes. A better understanding of the molecular mechanisms of early processes in plant defense responses, as provided by these studies, may in the long term help to develop environmentally safe plant protection methods for agriculture.

Introduction

Plants are resistant to most potential pathogens (non-host or species resistance). Only a limited number of pathogens are able to delay or prevent the onset of an efficient defense response of certain plant species and thereby constitute the relatively few true host/pathogen combinations (species susceptibility). Certain cultivars within such host plant species are highly resistant to specific races of these successful pathogens (cultivar resistance). The mechanisms of non-host and cultivar resistance appear to be similar. They involve preformed barriers as well as active defense reactions. Among the induced responses specific gene activation appears to play a central role. The activation of plant defense genes in the nucleus requires some type of recognition of the pathogen, probably at the plant cell surface, and subsequent transduction of a signal to the nucleus. Since successful resistance reactions in infected plant tissues are limited to a few cells at the infection site, the molecular events underlying the defense response are difficult to study in the intact plant. A number of experimental systems have therefore been developed in which whole populations of cultured plant cells respond almost synchronously to treatment with pathogen- or plant-derived elicitors. These model systems have been used to investigate plant defense gene activation and are now also employed to study the elicitor recognition and signal transduction processes.

Reprint requests to Dr. Dierk Scheel.

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Elicitors of plant defense gene activation

The term "elicitor" was originally used for compounds that induce the accumulation of phytoalexins in plants [1], but is now applied in a more general sense to substances stimulating any typical defense response [2, 3]. Many of these reactions rely on rapid transcriptional activation of specific genes which are classified as plant defense or plant defense-related genes. Typical examples include the genes encoding enzymes of phytoalexin biosynthesis [2–4], lytic enzymes, such as chitinases and glucanases [5], cell wall components, for example hydroxyproline-rich glycoproteins [6] and glycine-rich proteins [7, 8], and peroxidases [9, 10]. A number of genes of unknown function have been identified by differential cDNA screening

[11]. In addition, cDNAs have been isolated which correspond to "pathogenesis-related" (PR) proteins originally detected on polyacrylamide gels of protein extracts from plants infected with avirulent pathogens [12].

Most studies on the mechanism of plant defense gene activation have been performed with crude elicitor preparations and these have been invaluable for that particular purpose. However, they may contain a mixture of structurally different components with elicitor-active or elicitor-antagonistic properties. For the investigation of events related to recognition and consequent signal transduction, elicitor purification is needed. This requires a simple assay system for defense gene activation. Phytoalexin accumulation offers one such possibility, since it is the result of transcriptional activation of the genes encoding the corresponding biosynthetic enzymes in all systems studied so far. In addition, the transcriptional induction of the general phenylpropanoid pathway is part of the defense response of many or all plants [13]. Stimulation of this pathway can easily be detected by measuring the activity of the first enzyme, phenylalanine ammonia-lyase (PAL). Careful controls have to be included in this case, since PAL activity also increases in many other stress conditions [13].

Cruickshank and Perrin [14] were the first to apply phytoalexin accumulation to detect an elicitor from the fungus *Monilinia fructicola* and monitor its purification. They isolated a protein with an apparent relative molecular mass (M_r) of approximately 8,000 that stimulated phaseollin accumulation in french bean *(Phaseolus vulgaris)*.

More recently, a glycoprotein of M_r = 46,000 was isolated from culture filtrates of *Phytophthora parasitica* var. *nicotianae* by assaying its ability to elicit the accumulation of the sesquiterpenoid phytoalexin, capsidiol, in tobacco *(Nicotiana tabacum)* callus [15]. The elicitor activity resided in the protein part of the molecule, since it was destroyed by boiling or digestion with pronase, whereas periodate treatment had no effect. This glycoprotein appears to be a common extracellular component of *Phytophthora*, as it was also detected in *P. cactorum, P. cinnamomi, P. infestans, P. megasperma* f.sp. *glycinea* and *P. parasitica* var. *nicotianae*, but not in *Pythium ultimum* and *Fusarium oxysporum* f.sp. *pisi*.

Albersheim and coworkers have identified oligosaccharides with 3-, 6- and 3,6-linked glucosyl residues from the hyphal cell wall of *Phytophthora megasperma* f.sp. *glycinea* as potent elicitors of glyceollin accumulation in soybean *(Glycine max)* [16]. The smallest compounds retaining elicitor activity were a hexa-β-glucosyl glucitol and the corresponding hepta-β-glucoside [17, 18]. Only one of eight stereoisomeric forms of the hepta-β-glucoside examined possessed elicitor activity, demonstrating a high degree of structural specificity [19].

A glycoprotein of M_r = 42,000 has been purified from culture filtrates of the same fungus. It elicits the accumulation of coumarin phytoalexins and of mRNAs encoding plant defense genes in cultured cells and protoplasts from parsley *(Petroselinum crispum)* [20]. The elicitor completely retains its activity after chemical or enzymatic deglycosylation, but loses it upon digestion whith pronase or trypsin. Thus elicitor activity resides in the protein moiety. The glycoprotein is also a constituent of the fungal cell wall. Interestingly, mycelial cell walls of *Phytophthora nicotianae* var. *parasitica* and *P. parasitica* contain the elicitor but it is not detectable in *P. infestans, Alternaria carthami, Fusarium solani, Rhynchosporium secalis* and *Sclerotinia sclerotiorum* (Wolfgang Schulte, Jane E. Parker, and Dierk Scheel, unpublished results). This suggests that it is not unique to *P. megasperma* but is by no means common to all fungal cell walls.

A peptidoglycan of M_r = 67,000 was recently isolated from germ tube walls of *Puccinia graminis* f.sp. *tritici* which induces the accumulation of lignin-like compounds in wheat *(Triticum aestivum)* [21]. The purification of this elicitor was followed by measuring its ability to stimulate PAL activity.

All of the elicitors described appear to be common extracellular components of phytopathogenic fungi and as such are predestined to serve as signals of non-self recognition by plants. It is however impossible to define a structural or functional consensus for the few elicitors of plant defense gene activation identified so far. The question arises, therefore, whether different plants recognize the same or different constituents from a certain pathogen as elicitor.

Another class of phytoalexin elicitors are pectic polysaccharides (oligogalacturonides) released from plant cell walls [16]. These oligogalacturonides act alone as well as synergistically with patho-

gen-derived elicitors in a number of systems [16, 17, 22]. It will be interesting to see at which stage of signal perception or transduction the synergistic amplification of the defense response is triggered.

Elicitor recognition

Studies of cultured cells from soybean, parsley or potato *(Solanum tuberosum)* treated with elicitor preparations from *Phytophthora megasperma* f.sp. *glycinea* or *P. infestans* demonstrated that the three plants not only recognized different components of the crude elicitor preparations but were unable to respond to certain fungal components identified by the other plants [20]. For example, parsley recognized proteinaceous compounds from *P. megasperma* and carbohydrates from *P. infestans,* but did not respond to carbohydrates from *P. megasperma.* In contrast, these carbohydrates were active elicitors in soybean and potato, whereas neither plant responded to the proteinaceous elicitor preparations from *P. megasperma* which were active in parsley. Similar results were obtained by Ricci *et al.* when they treated carnation *(Dianthus),* pepper *(Capsicum annuum),* potato or tobacco with arachidonic acid or extracellular components from *P. capsici, P. cryptogea* and *P. parasitica* [23]. Soybean responded to polysaccharides, carnation to glycopeptides and the solanaceous plants to structurally different lipids. We therefore envisage the presence of different specific target sites for elicitor recognition on the plant cell surface, rather than a more general mode of elicitor action.

The presence on the plant surface of receptor-like binding sites for elicitors of phytoalexin accumulation has been postulated for some time [16]. These are probably not located in the cell wall, since in at least two systems freshly prepared protoplasts respond to elicitors in a similar way as the intact cells [24, 25]. Components of the cell wall may nevertheless be involved in signal modulation. The most likely site of elicitor perception is the plasma membrane and internalization of the elicitor as part of the recognition process can not be ruled out. Putative elicitor binding sites can be detected in binding assays utilizing membrane vesicles and radioactively labeled pure elicitor molecules which completely retain their elicitor activity. These types of experiment have been carried out to date with only three systems, soybean, wheat and parsley.

Ebel and coworkers isolated 1,3-, 1,6-β-glucan fragments within a narrow size range from *Phytophthora megasperma* which were highly active elicitors in soybean, and radioactively labeled these without loss of elicitor activity [26, 27]. In binding studies with membrane preparations from soybean roots, hypocotyls, cotyledons, and cultured cells, the ^{3}H- or ^{125}I-labeled β-glucans exhibited saturable and reversible binding with high affinity. In addition, protoplasts isolated from cultured soybean cells also showed competable β-glucan binding with an affinity identical to that found in the membrane preparations [27]. In competition experiments, a strong positive correlation was observed between the ability of structurally related oligosaccharides to compete for binding of the radiolabeled β-glucan to soybean membranes and their activity as elicitors of glyceollin accumulation in soybean cotyledons [26, 27]. The strongest competitor was a chemically synthesized hepta-β-glucoside which was identical in structure to the smallest elicitor-active β-glucan isolated from *P. megasperma* [28].

Applying similar techniques, binding sites for the peptidoglycan elicitor from germ tube walls of *Puccinia graminis* f.sp. *tritici* have recently been detected on wheat membrane vesicles [29]. The saturable and reversible binding was higher on plasma membranes than on intracellular membranes. The binding affinity of this ligand was approximately one hundred-fold lower than that of the β-glucans to soybean membranes, but the results obtained with a radiolabeled ligand were confirmed by ELISA experiments using polyclonal antisera against the elicitor.

Finally, preliminary experiments with the ^{125}I-labeled glycoprotein elicitor of furanocoumarin accumulation in parsley, isolated from *P. megasperma,* also indicated the presence of saturable binding sites on parsley plasma membranes (Jane E. Parker und Dierk Scheel, unpublished results).

Taken together, these results reveal the presence on the plasma membrane of specific receptor-like binding sites for extracellular components of fungal pathogens that elicit plant defense gene activation. Since a certain plant cell responds to elicitors of diverse structure with the activation of the same genes, the nucleus either responds to different sig-

nals from individual transduction chains initiated by different elicitor binding events, or signals originating from different binding sites are funneled through the same signal transduction mechanism. The two alternative models are shown in Fig. 1.

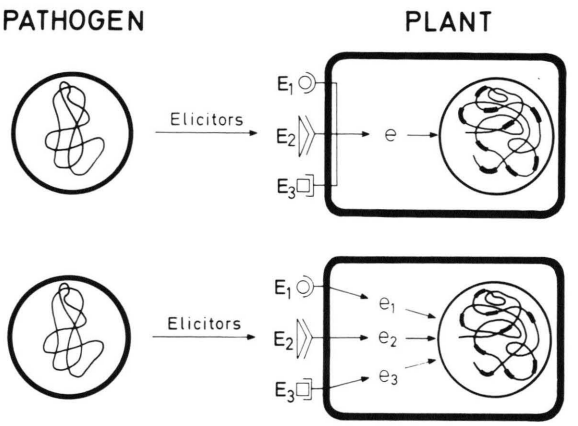

Fig. 1. Hypothetical models of alternative signaling mechanisms in plant defense gene activation. Structurally different elicitors (E_1, E_2, E_3) are released from pathogens or from plant cell walls. They bind to specific receptor-like structures on the plant plasma membrane. This event initiates either a unique chain of signals (e) or different, receptor-specific signaling cascades (e_1, e_2, e_3). In the upper model, diverse defense genes respond to the same signal, whereas in the alternative case different signals activate the whole set of genes.

Signal transduction

At present very little is known about transmembrane signaling in response to environmental stimuli in plants. The existing models rely on results obtained with animal systems. Recent studies suggest that key elements of some signaling cascades are also present in plants.

Convincing evidence has been obtained for the involvement of Ca^{2+} in elicitor-mediated defense gene activation. Omission of Ca^{2+} from the medium of cultured cells of carrot *(Daucus carota)* [30], parsley [20], potato [20] and soybean [31] significantly reduced the elicitor response assayed in the respective plant. In parsley protoplasts this was preceded by a reduction in the run-off transcription rates of plant defense genes, while transcription of other genes was unaffected [28]. Direct measurements of ion concentrations in the culture media of elicitor-treated parsley cells revealed decreases in Ca^{2+} levels, increases in K^+ concentrations and alkalization of the medium, all commencing within two minutes of elicitor addition. Using $^{45}Ca^{2+}$ an elicitor-mediated Ca^{2+} influx in cultured parsley cells was shown to follow a similar time course. In soybean [31] and carrot [30], but not in parsley [28], the Ca^{2+} ionophore, A 23187, stimulated phytoalexin accumulation. Patch-clamp analysis on single parsley protoplasts, which retain their responsiveness to elicitor [24], may elucidate if these ion fluxes are primary events in transduction of the elicitor signal or an indirect response to depletion of internal Ca^{2+} stores which are mobilized upon elicitation.

In animal systems, internal Ca^{2+} levels are often regulated by phosphoinositides [32]. All elements necessary for the operation of this second messenger system appear to be present in plants [33]. While in soybean and parsley phosphoinositide metabolism seems not to be involved in the response to elicitors [34], an elicitor-mediated increase in D-*myo*-inositol 1,4,5-trisphosphate was reported to precede 6-methoxymellein synthesis in cultured carrot cells [35]. With respect to the extremely complex metabolism of phosphoinositides in plants [33], more detailed analyses are required to confirm these results and clarify the precise signaling components.

Cyclic AMP, another important second messenger in animal sytems, is apparently not involved in signal transduction leading to defense gene activation in plants. In soybean [36] and parsley [20] concentrations of cAMP did not change in response to elicitors of phytoalexin accumulation.

The operation of any one of these second messenger systems would imply the involvement of protein kinases. Elicitor treatment of soybean cells prelabeled with radioactive phosphate, indeed resulted in changes in the pattern of phosphorylated proteins [37]. Recently, *in vivo* phosphorylation experiments with elicitor-treated parsley cells identified at least sixteen different proteins that were specifically phosphorylated in response to elicitor [38]. Interestingly, these phosphorylations were dependent on external Ca^{2+}, as were mRNA accumulation and transcription rates of plant defense genes [28, 38].

Altogether, the results described indicate the involvement of various ion fluxes and Ca^{2+}-dependent protein kinases in the intracellular transduc-

tion of elicitor signals in plant defense gene activation. A schematic drawing is shown in Fig. 2 which summarizes recent findings and includes some speculative suggestions, such as the participation of GTP-binding proteins. In addition, this diagram tries to bring together known pieces of the signaling network within a working hypothesis for future experiments.

Defense gene activation

The last step in transduction of the elicitor signal is the activation of the plant defense genes. Some of the phosphorylated proteins may well represent factors involved in the initiation of defense gene transcription. However, the network of phosphorylation and dephosphorylation reactions may be very complex. So far, the promoters of only a few plant defense genes have been analyzed for the presence of specific regulatory elements and no *trans*-acting factors have yet been identified.

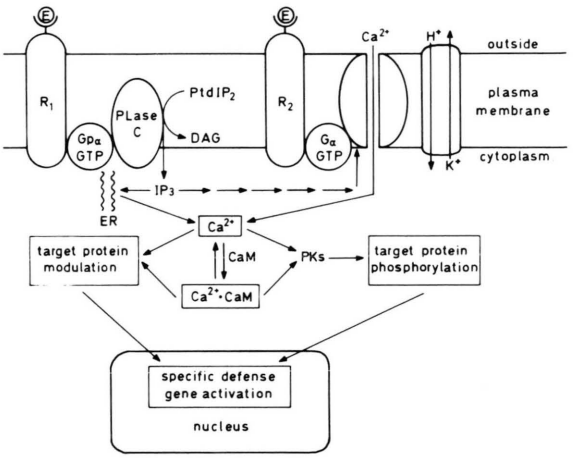

Fig. 2. Working hypothesis summarizing possible components involved in elicitor-mediated signal initiation and transduction leading to plant defense gene activation. Solid evidence has been obtained for the existence of elicitor-binding sites (R_1, R_2) on the plasma membrane, for the occurrence and the direction of Ca^{2+}, K^+ and H^+ fluxes through the plasma membrane and for the phosphorylation of microsomal, soluble and nuclear target proteins. Abbreviations: CaM = calmodulin, DAG = diacylglycerol, E = elicitor, G_α and Gp_α = α-subunits of specific GTP-binding proteins, IP_3 = D-*myo*-inositol 1,4,5-trisphosphate, PK = protein kinase, PLase C = phospholipase C, $PtdIP_2$ = phosphatidylinositol 4,5-bisphosphate.

A soybean protoplast system, retaining its responsiveness to elicitor, has been used to functionally analyze the chalcone synthase promoter [39]. Chalcone synthase (CHS) is involved in phytoalexin biosynthesis of french bean (*Phaseolus vulgaris*) [13]. The gene encoding this enzyme is transcriptionally activated by elicitor treatment of cultured bean cells [40]. A chimeric gene comprising the 5′-flanking region of the bean CHS gene fused to the coding region of a bacterial chloramphenicol acetyltransferase gene as reporter was responsive to elicitor treatment after electroporation into soybean protoplasts [39]. Analysis of 5′ deletions suggested the presence of elicitor-regulated activator and silencer elements in the CHS gene promoter.

In parsley the promoter of the PAL-1 gene was analyzed by *in vivo* footprinting experiments [41]. PAL is involved in the synthesis of phytoalexins and other phenylpropanoids in parsley and is transcriptionally induced by elicitor treatment of cultured cells [13]. Three putative elicitor responsive *cis*-acting elements were defined in the PAL-1 promoter, which showed striking homologies with sequences present in promoters of other genes encoding phenylpropanoid enzymes and/or stress-responsive genes [28]. UV-light inducible footprints were also detected within the conserved motifs of the parsley 4-coumarate:CoA ligase gene [41] which is coordinately regulated with PAL [13]. The activator element of the bean CHS promoter [39] mentioned above is located in a region that also contains the conserved elements [41]. The promoters of two other plant defense genes of parsley, PR 1 and PR 2, do not exhibit these sequences, but contain different elements mediating elicitor responsiveness (Iris Meier, Ulla van de Löcht, and Imre E. Somssich, unpublished results). Further analysis is required to characterize these sequence motifs and the corresponding *trans*-acting factors that may represent final components of the signal transduction chain.

Conclusions

Plants are able to recognize components of the microbial surface which act as elicitors of induced defense reactions. Results so far suggest that there is no consensus in elicitor structure in different plant-pathogen interactions. Indeed, the same

plant can recognize and respond to molecularly quite different elicitors. Further studies using purified, structurally well defined elicitor molecules may yet identify a common link in the mode of recognition. However, we suggest that plants have specific binding sites on their surface for several if not many structurally distinct elicitors.

Cultured plant cells and protoplasts provide a means to identify pathogen and plant components involved in the basic recognition process and the mechanisms of signal transduction leading to gene activation. The chain of transmembrane and intracellular events from signal perception to gene transcription is very fragmentary and operational models are based on data gathered from different plant systems. We keenly look forward to the elucidation of a complete signal pathway in one plant/pathogen interaction. Future experiments might also test whether multiple signals are generated from different elicitor binding events or a common signaling cascade is initiated. Finally, identification of the links between signal transduction and specific initiation of transcription requires a detailed functional analysis of plant defense gene promoters.

The mechanisms of non-host and cultivar resistance appear to be similar. However, the extent to which elicitors analyzed in cultured cell systems play a determinative role in the whole plant/pathogen interaction is not known. None of the elicitors described reflect the specificity of certain pathogen races towards a number of host cultivars. The isolation of such elicitors is only possible in systems where true gene-for-gene relationships have been established. This has recently been elegantly demonstrated in both a fungal [42] and bacterial/plant association [43]. The two elicitors were identified by their ability to induce necrotic reactions on resistant host tissue. It is not known if they also elicit defense gene activation. It will be of interest to see how race-specific and non-specific elicitors function in relation to cultivar and non-host resistance. Although the resistance responses appear to be similar in both cases it is still open whether the same or different signal transduction pathways are used.

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