Resistance response physiology and signal transduction
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Plants defend themselves against pathogen attack by activating a multicomponent defense response. The activation of this response requires recognition of the pathogen and initiation of signal transduction processes that finally result in a spatially and temporally regulated expression of individual defense reactions. Several components involved in signaling resistance reactions have recently been identified and characterized.

Introduction
Plants are resistant to most potential pathogens in their environment, as they are either not host plants for a particular pathogen species or are host plants, but harbor resistance genes allowing them to specifically recognize distinct pathogen races that carry the corresponding avirulence gene [1•,2•]. In both cases similar, if not identical multicomponent defense responses are initiated most probably by the interaction of elicitors that act as ligands for receptors in the plant plasma membrane or cytosol [1•,3•]. Although several resistance genes have been isolated, receptor function of the corresponding proteins remains to be demonstrated [2•]. In contrast, several putative non-host receptors have been detected, but with one possible exception [4•] the corresponding genes have not been isolated [1•]. Only fragments of the signal networks linking such receptors to defense response elements are known [1•,5•].

Resistance response physiology
Pathogen recognition at the site of infection initiates cellular and systemic signaling processes that activate multicomponent defense responses at local and systemic levels, and these responses result in rapid establishment of local resistance and delayed development of systemic acquired resistance [1•,6•,7•]. In a generalizing view that ignores species- and interaction-specific features, the earliest components of the cellular response include directed movements of organelles and the nucleus towards the site of pathogen attack [8–10], extracellular generation of reactive oxygen species (ROS) [11,12•,13•], formation of cell wall appositions mostly consisting of callose at the site of attempted penetration [12•], often followed by cellular collapse which is one type of programmed cell death called the hypersensitive response (HR) [14•]. These processes are frequently accompanied by release of phenolics from disintegrating cellular compartments, which upon contact with cytosolic enzymes are chemically modified or polymerized [15•]. Accumulation of defense gene transcripts follows these initial events sometimes in the attacked cells, but mostly in surrounding tissue [16•]. These genes encode pathogenesis-related proteins, such as glucanases, chitinases, defensins etc., and enzymes involved in the biosynthesis of phytoalexins and other, often phenylpropanoid- or fatty acid-derived secondary metabolites. Some of these products act directly as defense factors, for example some pathogenesis-related proteins and phytoalexins, whereas others apparently represent signaling elements, such as jasmonate and salicylate, some of which participate in the induction of systemic acquired resistance [6•,7•]. This latter type of broad resistance is accompanied by systemic development of microlesions and gene activation [17••]. In summary, plants are able to activate an efficient multicomponent defense machinery, if potential pathogens are timely recognized and the resulting signals are appropriately processed.

Signal transduction
Apparently, pathogens are recognized by perception of elicitors through receptors that are either located on the plasma membrane or in the cytosol [1•–3•]. Binding of the elicitor ligand to its receptor initiates a signal transduction chain, whose components have been most frequently studied with pharmacological methods in cultured plant cells treated with various elicitors. Many elicitors do not stimulate hypersensitive cell death, even when this reaction occurs during the corresponding plant–pathogen interaction [1•].

Calcium and ion channels
The earliest reactions of plant cells to elicitors are changes in plasma membrane permeability leading to calcium and proton influx and potassium and chloride efflux [1•]. These ion fluxes appear to be necessary and sufficient for induction of the oxidative burst, defense gene activation and phytoalexin production [18••,19••]. In parsley, a novel elicitor-responsive calcium inward channel has been detected and characterized [20••]. By binding to its receptor, the elicitor increases the open probability of this plasma membrane-located ion channel and may thereby stimulate elevated cytosolic calcium levels, as well as activate additional ion channels and pumps that cause the other ion fluxes observed. Another calcium-permeable channel was found to be activated in tomato protoplasts.
in response to a race-specific elicitor from Cladosporium fulvum [21]. As in parsley, open probability was increased by elicitor treatment, whereas single channel conductivity was unaffected. Transient increases in cytosolic calcium concentrations have been detected in tobacco cells in response to various elicitors [22*]. The inhibitory effects of different calcium channel inhibitors, calcium chelators and omission of extracellular calcium on these elicitor-stimulated transient changes in cytosolic calcium levels suggest that they are at least partially caused by calcium influx. The experimental evidence currently available, however, is also consistent with receptor-mediated release of calcium from internal stores as an immediate elicitor response, which then triggers calcium influx. Elevation of cytosolic calcium levels was also observed in epidermal cowpea cells of resistant plants, but not susceptible plants upon infection with appropriate races of the cowpea rust fungus [23**]. Calcium channel inhibitors preventing increases of cytosolic calcium concentrations also delayed the development of the hypersensitive response.

**G proteins**

Several lines of pharmacological evidence suggest that heterotrimeric GTP-binding proteins and protein phosphorylation/dephosphorylation are involved in transferring elicitor signals from the receptor to calcium channels that activate downstream reactions, such as the oxidative burst and phytoalexin accumulation [21,24,25*]. In cultured soybean cells, mastoparan, a G protein-activating peptide, was found to stimulate calcium influx, increases in cytosolic calcium levels and production of reactive oxygen species in the absence of elicitor [22*]. Mastoparan treatment of parsley cells activates ion fluxes, oxidative burst and phytoalexin accumulation in a manner comparable to that of the elicitor response (H. Zieheker, D. Scheel, unpublished data). Ectopic expression of the cholera toxin A1 subunit inhibiting GTPase activity of G proteins in tobacco plants resulted in high salicylate levels, constitutive expression of PR proteins and enhanced pathogen resistance [26]. Similar effects were reported for tobacco plants expressing a small GTP-binding protein [27].

**Reactive oxygen species**

Elicitor-mediated calcium influx, as well as transient elevation of cytosolic calcium levels were found to be necessary for elicitor stimulation of the oxidative burst [18**,22*]. This extracellular generation of ROS, such as superoxide, hydrogen peroxide and hydroxyl free radical, is a central component of the plants defense machinery [11,28]. ROS act as direct toxicants to pathogens [29], catalyze early reinforcement of physical barriers [30] and are involved in signaling later defense reactions, such as phytoalexin synthesis and defense gene activation [18**,31], programmed cell death [31,32] and protective reactions in healthy tissue against ROS damage [33].

Several lines of evidence suggest that ROS production during the oxidative burst is catalyzed by a plasma membrane-located NAD(P)H oxidase that might show some homologies to the mammalian respiratory burst oxidase [19*,25*,34,35,36*,37*]. As in mammalian phagocytosing cells, superoxide radicals are the first ROS generated and are then rapidly converted to hydrogen peroxide and oxygen, probably by extracellular superoxide dismutase [18**]. Furthermore, the occurrence of hydroxyl radicals has recently been described in elicitor-treated rice cells [38].

In several plant species, including parsley, the oxidative burst was found to be necessary for phytoalexin production [18**,39,40], whereas inhibition of elicitor-stimulated ROS production did not affect phytoalexin synthesis in soybean and tobacco [33,41,42]. Loss-of-function and gain-of-function experiments with cultured parsley cells demonstrated that superoxide radicals generated during the oxidative burst are involved in receptor-mediated induction of phytoalexin accumulation by an oligopeptide elicitor [18**]. Since superoxide radicals cannot cross the plasma membrane, they probably generate novel signals by interacting with plasma membrane components, such as fatty acids and proteins.

Superoxide radicals were also found to be necessary and sufficient to trigger programmed cell death and PR protein expression in Arabidopsis lsd1 mutants (lesion simulating disease resistance response) which are unable to control lesion spreading once it is initiated [31]. The corresponding gene encodes a zinc finger protein that monitors a superoxidase-dependent signal and negatively regulates plant cell death and defense gene activation [43**].

Infection of wild-type Arabidopsis plants with an avirulent strain of Pseudomonas syringae stimulates the formation of local and small-sized systemic lesions, as well as systemic acquired resistance (SAR) [17**]. Systemic lesion formation and SAR depend on hydrogen peroxide from the oxidative burst and on secondary systemic microbursts [17**], but the local reaction requires both, hydrogen peroxide and nitric oxide (M Delledonne, Y Xia, RA Dixon C Lamb, personal communication). Avirulent bacteria stimulate simultaneous formation of hydrogen peroxide and nitric oxide, whereas inoculation with virulent bacterial strains induces only a minor response. In cultured soybean cells, hydrogen peroxide and nitric oxide in combination are necessary and sufficient for defense gene activation and induction of programmed cell death. The authors propose the existence of a binary signal transduction system with separate pathways for ROS and nitric oxide generation that synergistically activate defense genes and programmed cell death (M Delledonne, Y Xia, RA Dixon, C Lamb, personal communication). In tobacco infected with tobacco mosaic virus, nitric oxide synthase (NOS) activity is increased and nitric oxide, as well as NOS application, induce PR protein synthesis and phenylalanine ammonia lyase expression (J Durner,
D Wendehenne, DF Klessig, personal communication). Pharmacological evidence from this experimental system suggests that, as in mammals, nitric oxide-mediated gene activation is signaled through cyclic GMP and cyclic ADP ribose. Cyclic ADP ribose, therefore, may represent a very upstream player in both hormone and defense signaling, since it activates ascobic acid- and pathogen-responsive genes through calcium release mechanisms (Jörg Durner, David Wendehenne and Daniel F Klessig, personal communication) [44**]. It remains to be elucidated, however, whether this calcium release is at all related to the calcium influx and transient increases in cytosolic calcium that are rapidly stimulated by elicitor treatment and infection [18**,20**,21,22**,23**].

**Protein kinases**

Pharmacological evidence and *in vivo* phosphorylation data suggest that phosphorylation cascades are involved in defense signaling at many different levels [32,45–51]. Some plant resistance genes encode receptor-like protein kinases themselves that may activate downstream signaling elements by phosphorylation [29,52,53]. The *Pto* gene of tomato, which confers resistance to bacterial speck disease, encodes a cytosolic serine/threonine kinase that interacts with other proteins, probably by phosphorylating them; some of these target proteins are putative transcription factors thought to activate PR protein-encoding genes [54*].

Recently, a class of protein kinases with strong homology to mitogen-activated protein kinases (MAP kinases) of yeast and mammals was found to be involved in signaling cascades of plants as well [55*]. Receptor-mediated rapid and transient activation of the elicitor-responsive MAP kinase, ERM kinase, was demonstrated in cultured parsley cells treated with an oligopeptide elicitor [56**]. Within the corresponding signal transduction chain, activation of this MAP kinase is located downstream of elicitor-responsive ion channels, but upstream or independent of the oxidative burst. Upon activation, ERM kinase was found to be translocated to the nucleus, where it may be involved in defense gene activation. Several putative transcription factors of plant defense genes indeed appear to be regulated by phosphorylation [57,58*,59].

A salicylate-responsive MAP kinase, SIP kinase, has been purified from tobacco [60**]. Salicylic acid, which is known to be an important signal molecule in local and systemic defense responses in many plants [5*], as well as its biologically active analogs, activate SIP kinase, induce PR protein expression and enhance pathogen resistance in tobacco [60**]. Furthermore, SIP kinase is activated in tobacco mosaic virus-infected resistant tobacco plants [61**] by two well-defined elicitors, parasitecin and cryptogein, and a crude cell wall elicitor which all derived from *Phytophthora* spp. [62**]. Most interestingly, the elicitors which activate defense genes, induce phytoalexin production and stimulate an oxidative burst and proton influx in tobacco, also cause hypersensitive cell death and prolonged SIP kinase activation. In contrast, the crude cell wall elicitor does not cause cell death and only activates SIP kinase very transiently. Furthermore, the elicitors activate at least two more unknown putative MAP kinases that do not respond to the cell wall elicitor preparation [62**].

A wound-responsive MAP kinase from tobacco, WIP kinase, is activated locally and systemically in response to tobacco mosaic virus infection in an resistance-gene dependent manner [61**]. In this case, the post-translational activation of WIP kinase is preceded by, and requires accumulation of, the corresponding transcript and protein. Since these processes are independent of salicylate, WIP kinase is considered to be a signal component located upstream of salicylate in local and systemic resistance of tobacco to tobacco mosaic virus. Differential activation of distinct MAP kinases at transcriptional, post-transcriptional and/or post-translational levels with elicitor-specific kinetics may, therefore, be important for the generation of signal-specific reactions. The importance of signal duration has also been observed for elicitor-stimulated ion fluxes in parsley, where quality and kinetics were found to be important for the resulting gene activation pattern [18**] and for the oxidative burst, which occurs with different kinetics in compatible and incompatible plant pathogen interactions [11]. Most interestingly, transcription factors were found to be differentially activated in animal cells by calcium response amplitude and duration [63**]. As elicitor-mediated ion fluxes appear to be located upstream of MAP kinase activation within defense signal cascades [56**], these regulation phenomena may be causally related.

**Jasmonate**

The rapid accumulation of jasmonate has been observed in many cultured plant cells in response to various elicitor treatments [1*,64*]. In suspension-cultured rice cells, an *N*-acetylaspartylglutamyl peptide elicitor induces the synthesis of the phytoalexin, momilactone A, which is preceded by transient accumulation of jasmonate [65]. Avoidance of elicitor-stimulated jasmonate accumulation by the lipoxygenase inhibitor, ibuprofen, also prevented phytoalexin accumulation which could still be induced by exogenous jasmonate application. Contrasting results were recently obtained with cultured parsley cells, where jasmonate accumulation is stimulated through the oligopeptide receptor downstream of ion fluxes and oxidative burst (T Kroj, D Scheel, unpublished data). In this experimental system, however, complete suppression of elicitor-stimulated jasmonate accumulation by the lipoxygenase inhibitors phenidone, indoprofen or ibuprofen does not at all affect elicitor-induced defense gene activation and phytoalexin accumulation. In accordance with this result, jasmonate or its derivatives are poor elicitors in parsley cells. It may be concluded, therefore, that jasmonate appears to be necessary and sufficient for triggering...
the defense response in rice, whereas it is at least not necessary in parsley.

Conclusions
The multicomponent defense response of plants to pathogens appears to be activated by ligand/receptor interactions, in which **avr** gene- and pathogen or plant surface-derived elicitors serve as ligands for plasma membrane-located or cytosolic receptors. Most elicitors activate defense genes and induce phytoalexin synthesis, whereas only a subset also stimulates programmed cell death. It is not clear yet, at which level of signal transduction both signaling pathways separate. Amplitude and duration, however, of individual signal elements may be important for the quality of the final reaction.

Highly conserved signaling elements appear to be employed in elicitor signal transduction, such as ion channels, calcium transients, protein kinases and phosphatases, G-proteins, ROS, NO, cyclic GMP, cyclic ADP ribose and fatty acid derivatives. The way how, and if at all, a distinct signal element is integrated into a given plants signaling network, however, appears to be rather variable.

Note added in proof
The papers referred to in the text as (M Delledonne, Y Xia, RA Dixon and C Lamb, personal communication) and (J Durner, D Wendehenne, DF Klessig, personal communication) have now been accepted for publication as [66**] and [67**] respectively.

References and recommended reading
 Papers of particular interest, published within the annual period of review, have been highlighted as:

- **of special interest**
- **• of outstanding interest**


This review clearly describes the differences between systemic acquired and induced resistance in plants.


The authors describe the light microscopic localization of reactive oxygen species in infected barley plants in relation to other early defense responses and programmed cell death.

13. Bestwick CS, Brown IR, Bennett MHR, Mansfield JW:
- The localization of reactive oxygen species in plant tissue infected with phytopathogenic bacteria is analyzed by electron microscopy and its appearance is correlated with programmed cell death.


A critical review on cell death in animals, fungi and plants.


17. Álvarez ME, Pennell RI, Meijer P-J, Ishikawa A, Dixon RA, Lamb C:
- The authors provide experimental evidence for a causal role of reactive oxygen species generated during the local oxidative burst in systemic development of microlesions, defense gene activation and resistance.


Evidence is provided that links receptor-mediated ion fluxes via the oxidative burst with defense gene activation and phytoalexin production.


An early response of tobacco cells to the elicitor cryptogein, is NADPH oxidation without changes in NAD+ and ATP levels.

- Receptor-mediated activation of a plant Ca**2+**-permeable ion channel involved in pathogen defense. Proc Natl Acad Sci USA 1997, 94:2751-2755.
- Patch-clamp analysis of an elicitor-responsive plasma membrane-located ion channel, whose open probability is altered through a receptor-mediated signal chain.


Changes in cytosolic Ca2+ levels are determined in aequorin-expressing soybean cells in response to various elicitors.


Changes in cytosolic Ca2+ levels are measured in epidermal cells of cowpea during compatible and incompatible interactions with rust fungi.


36. The plant homolog of a subunit of the human respiratory burst oxidase has been isolated, characterized and its localization in the plasma membrane determined using homologous antisera.


38. Structure and expression of six Arabidopsis genes with significant homology to a subunit of the human respiratory burst oxidase are described.


42. The gene encodes a protein that monitors a superoxide-dependent signal and negatively regulates plant cell death and defense gene activation.


55. Genes encoding putative transcription factors for defense genes interact with the cytoplasmic receptor for a bacterial avirulence gene product.


57. The author reviews evidence for functional involvement of MAP kinases in plant signal transduction pathways.


59. A MAP kinase is activated through an elicitor receptor and integrated into the corresponding signal transduction chain.

60. Yu LM, Lamb CJ, Dixon RA: Purification and biochemical characterization of proteins which bind to the H-box cis-


Elicitor treatment of soybean cells activates a protein kinase that phosphorylates and thereby activates a transcription factor of defense genes.


The authors have purified and characterized a salicylate-responsive MAP kinase and isolated the corresponding gene.


The rather unusual case is described that a MAP kinase involved in resistance gene-mediated signal transduction is activated at the transcriptional level.


MAP kinases are differentially activated by various elicitors and the duration of their activation is correlated with response specificity.


The differential activation of transcription factors in animal cells appears to be regulated through response amplitude and duration of Ca²⁺ transients, a regulatory mechanism that is probably also important in plants.


Direct evidence is provided for the involvement of both hydrogen peroxide and nitric oxide in signaling defense gene activation and programmed cell death in plant disease resistance.


Results are presented that suggest the involvement of a signal transduction chain consisting of nitric oxide, cyclic GMP and cyclic ADP ribose in elicitor-mediated gene activation.