Update on Plant-Microbe Interactions

Systemic Acquired Resistance

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One reaction of plants to pathogen infection is the induction of a long-lasting, broad-spectrum, systemic resistance to subsequent infections. This induced disease-resistance response has been known for many years under different names such as physiological acquired immunity or induced resistance; for the purpose of this update we will refer to it as SAR. Our perspective is that SAR is one component of plants' integrated disease-resistance repertoire. SAR appears to be distinct from preexisting resistance mechanisms such as physical barriers or protein cross-linking and also from other inducible resistance mechanisms such as phytoalexin biosynthesis, the hypersensitive response, and ethylene-induced physiological changes. Furthermore, SAR is not related to responses induced by wounding or osmotic stress. In this update we will provide a brief history of SAR research and review recent results important to our understanding of this response. We will also present a working model of the critical steps that lead to the induction and maintenance of the resistant state and point out steps in the response that need further experimentation to extend our understanding of SAR.

HISTORY OF SAR

For over 90 years, scientists and naturalists have observed that when plants survive pathogen infection they develop an increased resistance to subsequent infections. In 1933, Chester reviewed 200 publications describing a phenomenon he termed physiological acquired immunity (Chester, 1933). At that time, scientists believed they were investigating a phenomenon analogous to the immune response in mammals. In retrospect, at least three different processes were being called acquired immunity: viral cross-protection, antagonism (or biocontrol), and what we now refer to as SAR. During the 30 years following Chester’s review, many papers were published on the subject, but most of these were descriptive studies extending the earlier observations. The first systematic study of SAR was published by A. Frank Ross in 1961. Using TMV on local lesion hosts, Ross demonstrated that infections of TMV were restricted by a prior infection. This resistance was effective against not only TMV but also tobacco necrosis virus and certain bacterial pathogens. Ross coined the term “systemic acquired resistance” to refer to the inducible systemic resistance (Ross, 1961b) and “localized acquired resistance” to describe the resistance induced in inoculated leaves (Ross, 1961a). It is still unclear whether SAR and localized acquired resistance are aspects of the same response or distinct processes.

In the past 30 years, SAR has been demonstrated in many plant species and the spectrum of resistance has been broadened to include not only viruses and bacteria, but also many agronomically important phytopathogenic fungi (Kuč, 1982). However, our understanding of the biochemical events leading to the establishment of SAR had not progressed substantially until the past dozen years. In 1982, Kees Van Loo showed that the accumulation of a group of extracellular proteins called PR proteins correlates with the onset of SAR (Van Loo and Antoniw, 1982). Ray White demonstrated in 1979 that SA and certain BA derivatives could induce both resistance and the accumulation of PR proteins (White, 1979). As a result, SA was considered as a possible endogenous signal molecule (Van Loo and Antoniw, 1982). However, progress slowed through the 1980s and the involvement of PR proteins and salicylic acid in SAR was questioned. Recently, significant progress toward understanding SAR has been made with the application of molecular biology, genetics, and enhanced biochemical tools. Nevertheless, our knowledge is still rudimentary and future progress will depend on an even more aggressive use of modern biological methods.

WORKING MODEL OF SAR

As a framework for the discussion of SAR, we will provide our current working model of the resistance. This model is not intended to be complete; it simply serves as a scaffold on which to build and test hypotheses. As shown in Figure 1, SAR can be conceptually divided into two phases, initiation and maintenance. The initiation phase may be transient and includes all of the events that lead to the establishment of resistance. The maintenance period describes the quasi-steady-state resistance that results from initiation. These terms simply serve as operational definitions and are not meant to imply that the processes are distinct.

GENE INDUCTION CORRELATED WITH SAR

A useful approach in the study of a complex biochemical response like SAR is to identify easy-to-measure markers that precisely correlate with the biological process. In attempts to

Abbreviations: BA, benzoic acid; PR, pathogenesis-related; SA, salicylic acid; SAR, systemic acquired resistance; TMV, tobacco mosaic virus.
find markers for SAR, we and others have isolated many cDNAs that are expressed in uninfected tissue during SAR maintenance. Using the tobacco/TMV model system, we showed that steady-state mRNA levels from at least nine families of genes are coordinately induced in uninfected leaves of inoculated plants; we refer to these families collectively as "SAR genes" (Ward et al., 1991). The time at which SAR gene expression is first detected (about 6 d following inoculation) correlates well with the time at which resistance can be bioassayed. Moreover, abiotic agents that induce resistance, such as SA and 2,6-dichloroisonicotinic acid (Métrax et al., 1991; Ward et al., 1991), induce the same spectrum of SAR gene expression to levels comparable to those in SAR. Thus, expression of the SAR genes tightly correlates with the onset of the resistant state.

Along with being very reliable markers for SAR, some of the genes apparently have an active role in resistance. Once the cDNAs were isolated and the encoded proteins purified, several were shown to have either direct antimicrobial activity or enzymic activities suggestive of antimicrobial proteins. For instance, several classes of SAR genes encode β-1,3-glucanases and chitinases (for review, see Linthorst, 1991). Such glucanoendohydrolases have previously been shown to have antifungal activity in vitro (Linthorst, 1991). Another class of SAR genes encodes a Cys-rich group of proteins related to an intensely sweet-tasting protein called thaumatin (Linthorst, 1991). Thaumatin-like proteins are known to be active against fungi in vitro (Vigers et al., 1991; Woloshuk et al., 1991). Their activity appears to reside in the ability to disrupt membrane integrity, which is the basis for calling this class of proteins "permatins." A final group of SAR genes known to inhibit fungal growth is related to the PR protein known as PR-1. The PR-1 proteins have no known biochemical function, but appear to be widely distributed in angiosperms. PR-1-related proteins from tobacco and tomato have in vitro activity against Phytophthora infestans (Cohen et al., 1992).

Further support for the involvement of SAR genes in resistance comes from experiments in which cDNAs were expressed in transgenic plants. Transgenic tobacco and Brassica seedlings that express a chitinase from bean have been shown to be significantly protected against damping-off caused by Rhizoctonia spp. (Broglie et al., 1991). We have recently shown that high-level expression of PR-1 in transgenic plants results in reduced infection by two Oomycete pathogens, Peronospora tabaci (which causes the downy mildew disease known as blue mold) and Phytophthora parasitica (black shank disease) (Alexander et al., 1993). Thus, the findings that several of the SAR genes encode antimicrobial activities and that expression of certain SAR genes in transgenic plants imparts pathogen tolerance strengthen the case that these genes play a direct role in maintaining SAR.

Although SAR gene expression is best characterized in tobacco, other species that have been examined also induce genes systemically in response to pathogen attack. In some cases, e.g. Arabidopsis, those genes are related to a subset of the gene families seen in tobacco (UKnes et al., 1992). In other cases, such as cucumber, the genes appear to be distinct from the major tobacco SAR genes (Métrax et al., 1989). Conceivably, each taxonomic group of plants may have evolved its own set of SAR genes in response to evolutionary pressure from a specific spectrum of pathogens.

Considerable research activity is now directed toward identifying genes that are involved in plant-pathogen interactions from a variety of species. Undoubtedly, some of these will serve as SAR markers in these new systems. Our understanding of how the plant manifests resistance will progress using these genes in transgenic plant experiments. Moreover, with the availability of reliable markers, systematic studies of the SAR induction process become possible.

**SIGNAL TRANSDUCTION FOR SAR**

The first step in the development of SAR is the recognition of pathogen infection by a plant. Once the plant reacts to the pathogen, signals are released that trigger resistance in adjacent as well as distant tissues. Importantly, not all plant-pathogen interactions lead to SAR induction. Compatible interactions can lead to SAR induction; thus, the pathogen need not induce a gene-for-gene resistance reaction (Kuč, 1982). Currently there is no common denominator that can be used to group "inducing pathogens," and this area needs further clarification.

SA has been proposed as one signal leading to SAR because its concentration rises dramatically after a pathogen infection (Malamy et al., 1990; Metraux et al., 1990). The most compelling evidence that implicates SA as a signal in SAR comes from experiments using transgenic tobacco to express the enzyme salicylate hydroxylase, encoded by the nahG gene from Pseudomonas putida (Gaffney et al., 1993). This enzyme catalyzes the conversion of SA to catechol, which is not an active SAR inducer. The NahG-expressing plants do not accumulate SA in response to pathogen infection and are unable to induce an SAR response to viral, bacterial, or fungal pathogens.

These experiments implicate the direct involvement of SA in SAR signaling, but they do not address whether SA is the...
long-distance, phloem-mobile signal for SAR. However, experiments by Hammerschmidt and co-workers suggests that SA may not be a systemic signal. In this study SA and acidic peroxidase levels (encoded by an SAR gene in cucumber) were measured in various tissues of cucumber after removal of a leaf infiltrated with Pseudomonas syringae (Rasmussen et al., 1991). Surprisingly, the inducing leaf could be removed 4 to 8 h postinoculation, before significant SA accumulation, without preventing the systemic induction of either SA or SAR gene expression. Although SAR was not directly measured, this result suggests the existence of a systemic signal that is distinct from SA.

**BIOSYNTHESIS OF SA**

In higher plants SA has been proposed to be synthesized from trans-cinnamic acid to SA, via the intermediates ortho-coumaric acid or BA. As discussed by Ward et al. (1991), such a pathway provides a link between pathogen induction of phenylpropanoid biosynthesis and SAR signal production. Recently, Raskin and co-workers have further defined the SA biosynthesis pathway and the enzymes involved (Yalpani et al., 1993). The final step in SA synthesis is the conversion of BA to SA by benzoic acid 2-hydroxylase, a probable Cyt P450 enzyme. Moreover, benzoic acid 2-hydroxylase activity is induced approximately 10-fold by pathogen infection and is blocked by a protein synthesis inhibitor (Leon et al., 1993).

Thus, one apparent pathway for in vivo SA production appears to be the conversion of trans-cinnamic acid to BA followed by ortho-hydroxylation to SA. However, this does not exclude the possibility that other pathways for the biosynthesis of SA may exist, including via iso-chorismate or even via polyketide biosynthesis, as occurs in bacteria. Once synthesized, the fate of SA in the cell is not clear. Like other phenolics in plants, SA is rapidly conjugated to an O-glucoside. The role of this conjugate is not clear, but it has been reported to be inactive as an inducer of PR-1 in tobacco (Enyedi et al., 1992; Hennig et al., 1993). It seems likely that the conjugate may serve either as a storage form that can be hydrolyzed as needed or as an inactive form targeted for catabolism. Considering the important role that SA plays as a signaling component, its biosynthesis and catabolism are an area requiring further investigation.

**MECHANISM OF SA ACTION**

The mechanism by which SA induces gene expression is unknown. Klessig and co-workers have purified an SA-binding protein from tobacco that binds only SAR-active SA analogs. Recently, Chen et al. (1993) have isolated a cDNA encoding this SA-binding protein and found that it encodes a catalase isozyme. SA and active SA analogs specifically inhibited the protein's catalase activity. This discovery has led to the speculation that the action of SA may be mediated by catalase inhibition. Because catalase converts \( H_2O_2 \) to water and \( O_2 \), inhibition would result in accumulation of reactive oxygen species that may act as secondary messengers to induce SAR gene expression. Consistent with this idea, inhibitors of catalase unrelated to SA, as well as \( H_2O_2 \) itself, can induce SAR gene expression (Chen et al., 1993). For many years, groups working on oligosaccharide and protein elicitors have postulated the involvement of reactive oxygen species in signal transduction leading to plant defense responses (Chen et al., 1993). Conceivably, these pathogen-derived elicitors and plant-produced SA share some signal-transduction components. Alternatively, inhibition of catalase may have direct effects on the reduction of pathogen growth. Exactly how reactive oxygen species induce SAR gene expression as well as whether other receptors for SA exist should be areas of fruitful research.

**GENETIC APPROACH TO SIGNAL TRANSDUCTION**

One powerful method for the dissection of complex signal-transduction processes is the application of mutant analysis. Recently, SAR mutants have been isolated by screening ethyl methanesulfonylate-mutagenized Arabidopsis plants for gene expression (Lawton et al., 1993). Mutants that were unable to induce PR-1 mRNA in response to 2,6-dichloroisonicotinic acid treatment were called nim (no immunity). Mutants with high constitutive PR-1 gene expression were called cim (constitutive immunity). Several cim mutants have necrotic areas on the leaves even when they are grown under sterile conditions, a phenotype referred to as lsd (lesions simulating disease). Similar 'lesion mimic' mutants have been described in other species, especially maize, but have not been previously shown to express biochemical and molecular markers characteristic of pathogen infection. Further mutant analysis promises to reveal details of the pathway outlined in Figure 1, beginning with the plant-pathogen interaction and ending with the development of the resistant state.

**SUMMARY**

Clearly, SAR is an important component of disease resistance that contributes significantly to plant health. Understanding the biochemical and molecular basis for SAR may lead to the development of both low-usage-rate fungicides that act by stimulating natural disease resistance mechanisms and improved crop varieties. Furthermore, SAR will undoubtedly serve as a paradigm for environmentally regulated signal-transduction systems.

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**LITERATURE CITED**


Broglie K, Chet I, Holliday M, Cressman R, Biddle P, Knowlton

Chester KS (1933) The problem of acquired physiological immunity in plants. Q Rev Biol 8: 275–324


Ross AF (1961b) Systemic acquired resistance induced by localized virus infections in plants. Virology 14: 340–358


