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# Phenolic Compounds as Regulators of Gene Expression in Plant-Microbe Interactions

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Plants synthesize a wide variety of phenolic compounds in both root and shoot tissues during normal growth and development. These compounds are produced via the phenylpropanoid biosynthetic pathway and are building blocks for plant pigments (Ebel and Hahlbrock 1982) used for cell wall structure (Hahlbrock and Grisebach 1979), protection from ultraviolet light, and defense against pathogens (Dixon *et al.* 1983), and are possibly used to modify hormone action (Jacobs and Rubery 1988). The synthesis of plant phenolics can also be induced in response to hormonal and environmental stimuli such as ethylene (Ecker and Davis 1987), pathogen infection, and wounding (Lawton and Lamb 1987).

Recent studies have indicated that plant phenolic compounds induce catabolic genes in many microorganisms as well as genes required to form active associations with plants. *Rhizobium* and *Agrobacterium* typify this phenomenon, because the bacterial genes involved in the formation of nodules and the transformation of plants are induced by specific plant phenolic molecules (Peters *et al.* 1986; Stachel *et al.* 1985). Many microbes in the rhizosphere catabolize phenolic compounds secreted by plant roots. The regulatory genes involved in the catabolism of some phenolic compounds and the nodulation process show significant similarities suggesting a common evolutionary origin of these genes. Other microorganisms in association with plants may respond to specific phenolic compounds to interact with their respective host plants, but the genes involved in such interactions have not yet been identified.

The initial mechanism underlying communication between the plant and microbe is independent of the fate of the association, whether pathogenic or symbiotic. In any interaction, recognition is the primary event that involves signal perception and transduction, an alteration in the activity of regulatory genes followed by the induction of specific biochemical pathways. The products of these pathways act directly or indirectly as signals for the host, setting in motion a cascade of events, including chemotaxis, leading to pathogenic or symbiotic associations. The evolution of specificity for recognition of the inducer may serve to define an ecological niche and drive speciation. Deciphering this "language" may lead to an understanding of the basic path by which various pathogenic and symbiotic organisms have evolved their respective biological niches.



The importance of phenolic compounds in interspecies interactions in the rhizosphere is also shown by the observation that these compounds induce the proliferation of haustoria of parasitic plants (Steffens *et al.* 1982). Since mycorrhizae infection is affected by the same mutation in the host that prevents nodulation (Duc *et al.* 1989), it is possible that phenolic compounds also play an important role in early interaction with these widespread fungi which are highly beneficial to the plant.

**Phenolic compounds induce a variety of genes in plant-associated bacteria: Catabolic genes are induced by aromatic compounds.** Microbes in the soil encounter a wide variety of aromatic compounds that are largely products of plants. Certain microbes have evolved specific catabolic pathways enabling them to use these compounds as carbon or energy sources. In some cases, these pathways reside on transferable plasmids (Harayama *et al.* 1987). The catabolic pathways are induced by specific substrates of these pathways. For example, the β-ketoadipate pathway enables the microbe to use monocyclic compounds while the naphthalene pathway enables the use of dicyclic compounds. The genes of the β-ketoadipate and naphthalene catabolic pathways, which reside on endogenous TOL and NAH7 plasmids of *Pseudomonas putida*, respectively, have been extensively studied. The organization and structure of the genes in the two pathways are very similar, suggesting that they have evolved from a common group of genes (Harayama *et al.* 1987).

There are two operons for each pathway. The two operons on the TOL plasmid are controlled by two regulatory genes. The first operon encodes the enzymes that convert aromatic compounds to benzoic acids. The enzymes encoded by the second operon convert benzoic acids to tricarboxylic acid cycle intermediates. Induction of the first operon requires the regulatory gene, *xylR*, and the substrate toluene (Ramos *et al.* 1986). Toluene and *xylR* also induce synthesis of a second regulatory gene, *xylS*. Transcription of the second operon is dependent on the *xylS* gene product, but independent of any inducer (Inouye *et al.* 1987). In the case of the NAH7 plasmid, the first operon of the pathway is expressed constitutively at a low level and is induced by the end product, salicylate, in an *nahR*-dependent manner (Schell 1985). The second operon is also induced by salicylate and *nahR*.

The β-ketoadipate pathway is also present in other soil microbes that actively interact with plants. This pathway is inducible in *A. tumefaciens, R. fredii, R. meliloti, R.*

*leguminosarum* bv. *viciae*, and *R. l.* bv. *trifolii*, but is constitutively expressed in *Bradyrhizobium* (Parke and Ornston 1986). Such widespread catabolism of plant phenolics by microbes with diverse ecological niches suggests that these pathways evolved a long time ago. The regulatory genes that recognize plant phenolics for these catabolic pathways appear to have been recruited to control genes involved in specific plant-microbe interactions.

**Monocyclic compounds induce *Agrobacterium* virulence genes.** Plant phenolics induce gene activities in *A. tumefaciens* leading to the transfer of genetic information from the bacteria to the plant. Stachel *et al.* (1985) identified monocyclic plant phenolic compounds that induce expression of several virulence genes of *A. tumefaciens*. Induction was found to depend on the structure of the phenolic compound, acetosyringone showing the greatest activity. Several compounds related to acetosyringone were also found to be effective inducers (Bolton *et al.* 1986), including some chalcones (Spencer and Towers 1988). The compounds that induce virulence genes are not normally made by the plant, but are produced upon wounding, a prerequisite for plant transformation by *A. tumefaciens*. It is not known whether these compounds are synthesized *de novo* or produced as breakdown products of existing plant phenolics. These compounds are derived from the common portion of the phenylpropanoid pathway from which phytoalexins are synthesized. This suggests there is an evolutionary relationship between plant defense response, synthesis of toxic phenolic compounds, and the ability of bacteria to use these compounds to establish an active association.

Recent experiments demonstrate that some genes required for DNA transfer from *A. tumefaciens* can be complemented by bacterial conjugation functions (Buchanan-Wollaston *et al.* 1987). The implication is that the ability of *Agrobacterium* to transfer DNA to a plant evolved from existing genes of bacterial mating by acquiring a new regulatory circuit which responds to plant wound signals. This raises the question of whether bacterial conjugation is regulated by genes analogous to the *Agrobacterium* virulence genes. Indeed a one-to-one correspondence of bacterial conjugation functions to *Agrobacterium* virulence functions has been proposed by Stachel and Zambryski (1989). In this analogy, *oriT* (*ori*, origin of replication) corresponds to T-DNA borders, *mob* (*mob*, plasmid mobilization function) corresponds to virulence genes *virD1* and *virD2*, and *tra* (*tra*, plasmid transfer function) is similar to *virE*.

**Flavonoids induce nodulation genes in *Rhizobium*.** Flavonoids, a special class of plant phenolics, induce *Rhizobium* nodulation genes that are required for symbiotic association with legumes. Induction of the nodulation (*nod*) genes is dependent on *nodD* (Mulligan and Long 1985). Compounds that induce nodulation genes have been isolated from plant exudates, and the chemical structure of these compounds is found to be host-symbiont specific (Rossen *et al.* 1987). In alfalfa, inducing compounds have been shown to be exuded only from the portion of the growing root with emerging root hairs (Peters and Long 1988), the same zone of the root that is developmentally responsive to *Rhizobium* infection (Bhuvaneswari *et al.* 1981).

Most recently, it has been found that a chalcone induces nodulation genes at a 10-fold lower concentration than does luteolin and that this chalcone is exuded from alfalfa roots (Maxwell *et al.* 1989). Some phenolic compounds also inhibit nodulation gene induction by the normal inducer. Compounds exuded from roots showed both inducing and inhibitory activities (Firmin *et al.* 1986; Djordjevic *et al.* 1987); however, the inhibitory activity of the exuded compounds has not been shown to play a role in nodule development. Flavonoids have been shown to be rate limiting for nodule formation in some hosts. Kapulnik *et al.* (1987) found increased nodule number, nitrogen fixation, and plant mass in alfalfa after the addition of luteolin to the plant growth medium. In addition, breeding experiments showed a strong correlation between increased nodulation and a higher level of luteolin synthesis. These data suggest that production of specific flavonoids and their release from roots are two of the important factors in the symbiotic interaction with *Rhizobium*.

**Soil microbes are chemoattracted to many plant phenolics.** Compounds known to be inducers of catabolic genes and genes involved in plant-microbe interactions act as chemoattractants (Harwood *et al.* 1984). For example, *Bradyrhizobium* and *R. l.* bv. *trifolii* are chemoattracted to benzoate and toluate. This chemotaxis requires prior exposure of the microbe to the compounds implying that some part of the sensing and signal transduction must be induced in the microorganism (Parke *et al.* 1985). *Agrobacterium* is chemoattracted toward inducers of virulence genes. This chemoattraction is independent of the presence of the tumor-inducing (Ti) plasmid, suggesting a chromosomal location of the genes involved in this process (Parke *et al.* 1987). However, Ashby *et al.* (1988) have indicated that a Ti plasmid-derived function is responsible for the chemoattraction. The apparent difference between the two observations may be due to the use of two different strains. Several mutants of *A. tumefaciens* have been isolated that are defective in chemotaxis (Hawes *et al.* 1988), indicating involvement of several genes in this process. *Rhizobium* may be similarly chemoattracted toward flavonoids (Armitage *et al.* 1988; Caetano-Anolles *et al.* 1988a). Since nonmotile mutants of *Rhizobium* are fully competent to form nodules, chemotaxis is not required (Caetano-Anolles *et al.* 1988b), but may provide a competitive advantage (Ames and Bergman 1981).

**Specificity resides in the regulatory genes.** Recognition of the inducer by regulatory proteins is the primary step in gene activation. Catabolic enzymes can only be used if the specificity of the regulatory proteins matches the specificity of the enzymes themselves. Recognition can limit host range and catabolic functions as has been demonstrated for both the degradation of halogenated phenolics by *P. putida* and nodulation by *Rhizobium*. In the case of *P. putida*, a strain carrying the TOL plasmid was found to be unable to degrade certain halogenated aromatic compounds. Upon further examination, it was found that the particular halogenated compounds did not induce the

catabolic enzymes. A regulatory protein able to respond to the halogenated compounds was selected by mutagenesis. When the gene encoding this mutated regulatory protein was inserted into *P. putida* containing the TOL plasmid, the halogenated compounds were catabolized (Ramos *et al.* 1986). This demonstrates that a microbe's metabolism can be restricted by regulatory proteins even though the metabolic enzymes have a broader substrate specificity.

Although there is no direct biochemical evidence, there is genetic evidence that flavonoid specificity in *Rhizobium* is conferred by NodD (Spaink *et al.* 1987). Analogous to the catabolic regulatory pathways, the nodulation genes are not expressed in the absence of the host plant. Nodulation gene induction by phenolic compounds triggers a series of events that allows the nodulation process to commence. Molecular recognition of inducing compounds by the NodD regulatory protein is the primary determinant of the host range of *Rhizobium*. After induction of the nodulation genes, other genetic loci can affect the host range. The host range of a particular *Rhizobium* spp. can be both extended (Horvath *et al.* 1987; Bassam *et al.* 1986) and restricted (Spaink *et al.* 1987) by substituting heterologous *nodD* genes that recognize different inducing molecules. Thus, the host range of *Rhizobium* is greatly affected by the specificity of the regulatory gene toward compounds from the root exudate. This could influence the competitiveness of *Rhizobium* in the rhizosphere. *Rhizobium* with narrow specificity of inducers may compete better on a given host than *Rhizobium* with a broad specificity, thus allowing selection of a species along with its host (Verma and Stanley 1989).

NodD has been functionally divided into two domains. The amino terminus of the protein is involved in the autoregulation of its synthesis (Burn *et al.* 1987; Spaink *et al.* 1989), and the carboxy terminus determines flavonoid specificity (Burn *et al.* 1987; Horvath *et al.* 1987; Spaink *et al.* 1989). Thus, the amino termini of NodD are well-conserved while the carboxy termini differ between species, providing flavonoid specificity. NodD sequences of *Rhizobium* are found to have sequence similarity to the *Pseudomonas* NahR regulatory protein (Schell and Sukordhaman 1989). This similarity is more prominent in the amino terminus. The similar regulatory properties, sequence homology, and recognition of phenolic compounds suggest a recent evolutionary divergence of these two transcriptional activator proteins.

Flavonoids also induce genes of *Rhizobium* of unknown function. It will be interesting to study the mechanism and control of this induction and how it differs from the control of nodulation genes induced by the same inducer. Two such genes from *R. fredii* (Sadowsky *et al.* 1988) have a conserved sequence in their promoter region, but showed no homology with nodulation gene promoters. Since these genes are induced by flavonoids but have no effect on nodulation, they may be involved in the catabolism of the flavonoid compounds.

**Signal transduction of phenolic compounds is similar to other bacterial regulons.** Two distinct classes of regulatory proteins in bacteria have recently been recognized. Ronson *et al.* (1987) noted a two-component regulatory system. Some members of this group are OmpR/EnvZ, NtrC/NtrB, PhoB/PhoR, and NifJ/NifL (David *et al.* 1988). In this regulatory model, one component serves as a sensory molecule and is usually membrane-bound; the other is the regulatory molecule that activates transcription. Transduction of the signal from the sensory component to the regulatory component is mediated by phosphorylation of the regulatory component by the sensory component in response to an environmental signal. This model is consistent with the regulation of virulence genes of *A. tumefaciens*. The second regulatory model is a single component system (Henikoff *et al.* 1988). This latter family of regulatory proteins is known as the LysR family. In this model, the regulatory protein binds to the promoter regardless of the presence of the inducer. The regulation of the nodulation genes of *Rhizobium* and the *xyl* and *nah* genes of *P. putida* is consistent with this model.

The regulation of virulence genes in *A. tumefaciens* is a two-component regulatory system. The induction of the virulence genes by plant phenolics requires functional *virA* and *virG* genes. VirA has been localized to the inner membrane (Leroux *et al.* 1987), and VirG has sequence homology with other bacterial activator proteins (Winans *et al.* 1988). VirA is the sensory component while VirG is the regulatory component. In the presence of phenolic inducers, VirA is an autokinase and phosphorylates the regulatory component, VirG (E. W. Nester, personal communication). VirG activates transcription of the Ti virulence genes by binding to the *vir*-box (Winans *et al.* 1988). The *vir*-box is defined as a 12 base pair sequence (TNCAATTGAAAY) that appears in the promoter region of all inducible virulence genes. It must be noted that virulence gene induction can also occur without plant phenolics, such as by starvation for phosphate and a weakly acidic pH (Winans *et al.* 1988). The latter is dependent on a chromosomal gene, *chvD*. This may extend the situations in which *Agrobacterium* is able to transfer DNA to plants.

Regulation of the nodulation genes by NodD appears to occur in the manner of the LysR family. Flavonoids are not required for binding of the *nodD* gene product to the *nod*-box (Hong *et al.* 1987; Fisher *et al.* 1988). The *nod*-box is a 25 base pair conserved sequence that precedes inducible nodulation genes (Rostas *et al.* 1986). Mulligan and Long (1989) have recent evidence that the nodulation genes may be activated by regulatory proteins which do not respond to flavonoids and may behave similarly to the NahR and NahS regulatory proteins of *Pseudomonas*.

**Conclusion and overview.** Plants exude phenolic compounds into the rhizosphere. Microbes have evolved to recognize these compounds as signals for active interaction. Both *Agrobacterium* and *Rhizobium* recognize the same metabolic intermediate, chalcone, to activate genes involved in their particular interactions with plants. Microbes probably first developed the capacity to catabolize these compounds for carbon and nitrogen requirements. As one tries to decipher the function of the nodulation genes, it may be instructive to keep in mind that these genes may have been recruited from catabolic or metabolic enzymes which would have been the first

response of the soil microbes to these compounds as they were encountered in the rhizosphere. The fact that the same compound, for example daidzein, induces nodulation genes as well as completely unrelated genes in *R. fredii* (Sadowsky *et al.* 1988) is an indication of the possible evolution of such a common mechanism. Characterization of such promoters may shed light on the possible common mechanism underlying this induction phenomenon. Although these phenomena have been studied in *Agrobacterium* and *Rhizobium*, phenolics may play a more general role in interspecies interaction as is evident from the induction of haustoria in parasitic plants. It will be interesting to find other examples of phenolics affecting specific genes in plant-associated microbes including mycorrhizae. Such experiments are now possible because more plant-interacting fungi can be transformed and their genes tagged.

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