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Review

Plant gene expression in response to pathogens

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Introduction

Many molecular biologists are turning their attention to the interactions between plant pathogens and their hosts. These offer some promising model systems for investigating the control of gene expression in plants and, of course, offer promise for the future genetic engineering of crop plants to incorporate desirable traits such as disease resistance. Similarly, many plant pathologists are adopting the techniques of molecular biology to solve problems that have proved intractable by conventional biochemical and physiological methods of investigation. The problems encountered by both groups are somewhat different and we hope that this article will be of interest to all in the field by providing a review of current work, while attempting to explain many of the specialist terms involved.

A note on terminology

Most of the terms explained here are taken from the booklet "A guide to the use of terms in plant pathology" [80], and Cooper and Jones [43].

A *pathogen* is an organism or virus able to cause *disease* (i.e. a harmful deviation from normal physiological function) in a host or range of hosts.

Resistance is the ability of the host to suppress or retard the activity of a pathogen and can take many forms [90]. Resistance is a quantitative property and is best considered in relation to the *virulence* of the pathogen. In plant pathology, the term virulence describes two concepts. Firstly, it describes the degree of *pathogenicity* (i.e. the "aggressiveness" of the pathogen), a definition of the term which refers to the severity of the disease caused by the pathogen; this is also the meaning of the term in animal pathology. However, in plant pathology, virulence has another, more specific meaning in relation to host range. When two physiological variants of a pathogen cause different reactions in the same host cultivar, one leading to disease and the other not, they may be classified as different races of the pathogen, leading to the term *race-specific resistance*. One race (causing disease) is said to be *virulent* on the cultivar whereas the other is described as *avirulent* (or better *non-virulent*, to avoid confusion when speak-

ing the terms). When a pathogen of a particular host plant species comes into contact with a different species on which it does not normally cause disease, the type of resistance expressed is called *non-host resistance* and the organism is said to be *non-pathogenic* on that host. Thus, when used in this way, the term *non-pathogenic* is synonymous with the term *avirulent* for race-cultivar interactions.

The interaction between a virulent race and a susceptible cultivar can be described as *compatible* whereas the interaction between a non-virulent race and the resistant cultivar is described as *incompatible.* These concepts are illustrated in Fig. 1.

Classical genetics has shown that the resistance trait in a cultivar showing race-specific resistance is often (though not always) inherited in a simple Mendelian fashion as though it were conditioned by single dominant genes. These are the *resistance genes* often met with in the literature. The inheritance of virulence and avirulence has also been studied in the pathogen [53]. This has been done for *Melampsora lini*, the Basidiomycete fungus causing flax rust [81] and for *Bremia lactucae*, the Oomycete fungus causing downy mildew of lettuce (*Lactuca sativa* [47]). In these cases virulence was found to be recessive to avirulence and both traits were inherited in a simple Mendelian fashion.

The finding that correlated variation occurred in host and pathogen, where a gene for resistance in the host corresponded to alleles of a gene in the pathogen, led to the concept of a *gene-for-gene* interaction [81]. A selection of cultivars carrying different resistance genes can be used to identify a number of *physiological races* of the pathogen. The way in which the races are defined differs in different host-pathogen interactions and this should be made clear to avoid confusion. For example, in the interaction between potato (*Solanum tuberosum*) and *Phytophthora infestans*, a race 1 isolate of the pathogen is virulent on potato varieties carrying the R_1 resistance gene;

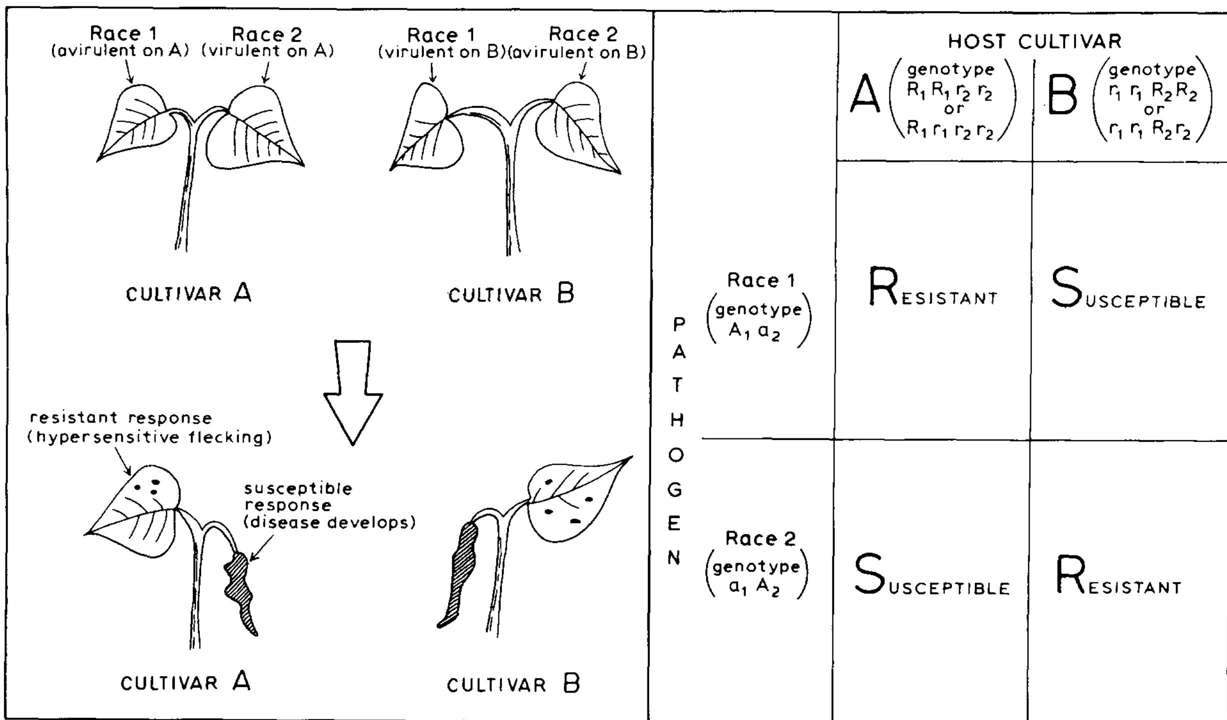


Fig. 1. Schematic representation of compatible and incompatible interactions between two differential cultivars of a host and two physiological races of a bacterial plant pathogen. Cultivar A has a resistance gene (R_1) to race 1 (which has the corresponding avirulence gene A_1). Race 2 lacks the avirulence gene present in race 1 and causes disease in cultivar A. The reverse is true for cultivar B which has a resistance gene (R_2) to race 2 isolates of the pathogen (which carry avirulence gene A_2). The table shows the reciprocal check nature of the interaction and lists the postulated genotypes of host and pathogen.

i.e. race 1 is defined by its apparent lack of the corresponding avirulence gene. Complex races of pathogens are those virulent on cultivars with more than one major resistance gene. For example, race 1,3,5 of *P. infestans* is virulent on those cultivars which have the resistance genes R_1, R_3 and R_5. All races of the pathogen cause disease on those cultivars which have no major genes for resistance. This is also how the races of *Cladosporium fulvum* (*Fulvia fulva*) are defined in their interactions with tomato, *Lycopersicon esculentum* [112]. In contrast, races of plant pathogenic bacteria have been defined on the basis of the avirulence genes they carry; thus, in the interaction of soya bean (*Glycine max*) with *Pseudomonas syringae* pv. *glycinea*, race 6 of the pathogen carries the race 6 avirulence gene [187]. The races of *Xanthomonas campestris* pv. *malvacearum* are similarly defined [89].

Caution must be exercised before assigning a particular host-pathogen combination to the gene-for-gene category, since pronouncements on genotype are often made without a thorough study of the genetics, but on simple analysis of phenotype, in this case the outcome of an interaction between host and pathogen. Once the inheritance of resistance has been established in Mendelian terms, physical study of the genes concerned through the use of molecular techniques should clarify the situation. A schematic representation of a hypothetical gene-for-gene interaction is illustrated in Fig. 1.

Since resistance and avirulence are dominant traits, it is generally believed that they are conditioned by positively acting gene products which perhaps interact as part of a recognition system [48, 54, 123]. Recognition, either directly or indirectly via second messengers, brings about changes in the metabolism of the host which lead ultimately to resistance [181]. Many of these changes are at the level of transcription or translation and these form the main subject of this review.

Associated with race-specific and non-host resistance is the *hypersensitive response (HR)*. This term is descriptive and implies a reaction of greater rapidity and intensity than that observed in compatible interactions, where, although the final levels of damage caused by the pathogen are much greater, the time taken to reach this stage is much longer than in HR. The term *normosensitive* [126] is used to describe the events seen in the susceptible reaction and contrast them to HR. HR can best be described as a rapid localised necrosis associated with limitation of pathogen spread. Complications arise if one has to define what is meant by necrosis and precisely when it occurs [145]. Indeed, the dead cells merely indicate that HR has occurred. What appears to be important is that the cells are dying in a coordinated way, which is associated with a characteristic set of changes in gene expression [41, 51, 100, 101, 144, 182, 197] and that this *de novo* protein synthesis is required by the cells in order to die [121]. This organised cell death may regulate gene expression in surrounding healthy cells (see the section on HR). In interactions with plant pathogenic viruses, HR does not always result in inactivation of the pathogen and the virus may spread beyond the necrotic lesion. In contrast, localisation of the virus may occur in the absence of any visible symptoms [43].

Associated with the HR are many of the induced, active resistance mechanisms discussed in this article. One of the most extensively studied of these is the production of the antimicrobial substances called *phytoalexins*. Phytoalexins are produced by the plant, are of low M_r, and are synthesized from remote precursors via biosynthetic pathways, the enzymes for which arise through *de novo* gene expression induced in the resistant reaction. The latter definition is meant to distinguish phytoalexins from antimicrobial compounds produced constitutively by plants, such as alkaloids, and those which arise by modification of a near precursor, e.g. hydrolysis of cyanogenic glucosides or glucosinolates which release cyanide or thiocyanata, respectively [4, 42, 154]. These latter responses are considered passive and so will not be discussed in this review because there is no evidence that they involve *de novo* gene expression induced by the pathogen.

Certain substances of biotic and abiotic origin called *elicitors* will induce some defence responses [58], for example the production of phytoalexins [61, 120], lignification [162] and pathogenesis-related proteins (PR or B proteins) [193] and browning associated with HR [3]. Abiotic elicitors include mercuric ions, polyacrylic acid and salicylate [15, 58, 193]. Biotic elicitors include fungal cell wall compo-

nents and substances present in culture filtrates. Keen [120] proposed that specific elicitors might be responsible for the specificity observed in the interactions between the host and different races of the pathogen. Race-specific elicitors of necrosis and chlorosis have been isolated from tomato leaves infected with *Cladosporium fulvum* [59]. One of these elicitors, the putative product of the A9 avirulence gene, was shown to be a peptide containing 27 amino acid residues (de Wit, pers. commun.). Glycoprotein elicitor molecules which mimic the behaviour of the parent *Phytophthora megasperma* f.sp. *glycinea* races have been reported [120, 122]. However, in the latter case, non-specific elicitors, which were more active than the race-specific elicitors, could be isolated from the pathogen or the medium in which it had been cultured. This led to the suggestion that race-specificity might be controlled by *specific suppressor* molecules produced by the pathogen which suppress the action of the non-specific elicitors. Some evidence for the existence of race-specific suppressors in the *Phytophthora infestans/Solanum tuberosum* interaction has been presented [66, 67]. However, this view does not easily fit classical genetical analyses which suggest that avirulence is dominant to virulence since production of suppressor (leading to virulence) would be expected to be dominant to non-production (leading to resistance). There is recent evidence that avirulence is indeed dominant to virulence in *P. infestans*, however, crosses are still being carried out to give information on all the loci involved (Al-Kherb, Shattock and Shaw, pers. commun.). Suppressors which act at the level of host-species and which establish basic compatibility between the host and pathogen have been reported [109, 110]. It was envisaged that the level of specificity seen in interactions between physiological races of the pathogen and different cultivars of the host was superimposed on this basic compatibility between host and pathogen [109, 110].

The above is by no means an exhaustive treatment of plant pathological terms but includes most of the expressions to be met with in the literature dealing with plant gene expression in response to pathogens or elicitors. One should perhaps add that some authors have implied slightly different meanings for these terms [43, 90] and the need for a reappraisal of certain expressions in the light of contemporary thinking may be appropriate.

Gene expression in plant defence responses

Two complementary strategies have been used with success to investigate pathogen-induced plant gene expression. Initial use of model systems, such as elicitor-treated cell culture or elicitor treatment of intact plant tissue, has provided much information on plant responses to pathogens which in general has been confirmed by subsequent studies of interactions of pathogens with intact plants.

(1) The targeted approach

A phenomenon correlated with the expression of disease resistance is identified. Enzymic or other proteins involved in the response are isolated, enzyme activities or effects on pathogen growth are measured and antibodies are raised to the protein(s) involved. These antisera can be used to measure changes in selected mRNA activities by immunoprecipitation from *in vitro* translations [20, 21, 26, 37, 46, 105, 115, 132, 135, 139, 152, 158, 169, 195] and as an aid to isolating cDNA clones of the enzymes involved [75, 115]. Once these probes are available, they can be used to determine specific gene activity by probing RNA "dot blots" and "Northern" blots, and run-off transcription of the particular gene can be measured [21, 38, 44, 46, 75, 116, 132, 169, 186]. Biochemical defence responses for which there is evidence for *de novo* gene expression are summarised in Table 1.

(2) The shotgun approach

The second approach is to study the expression of plant genes during interactions with pathogens without any preconceptions about their functions. Generally, the approach has been to use *in vitro* translation to follow changing patterns of mRNA activity on treatment of plant tissue with elicitor or exposure to a potential pathogen [41, 51, 100, 101,

Table 1. Host gene expression following interactions with plant pathogens and elicitors[1]

Genes induced	Species and tissue	Pathogen or elicitor	Ref.
	Phenylpropanoid metabolism		
Phenylalanine ammonia-lyase (PAL)	*Phaseolus* hypocotyls	*Colletotrichum*	[20, 45, 46]
	Phaseolus leaves	*Pseudomonas*	[*]
	Phaseolus culture	Elicitor	[26, 135]
	Pisum endocarp	Elicitors	[139]
	Glycine culture	Elicitor	[72]
	Glycine hypocotyls	*Phytophthora*	[76]
	Petroselinum culture	Elicitor and UV	[38, 132]
4-coumarate CoA-ligase (4CL)	*Petroselinum* culture	Elicitor and UV	[38, 66, 132]
Chalcone synthase (CHS)	*Phaseolus* hypocotyls	*Colletotrichum*	[20, 26]
	Phaseolus culture	Elicitor	[20, 169]
	Phaseolus leaves	*Pseudomonas*	[*]
	Petroselinum culture	Elicitor and UV	[38]
	Glycine culture	Elicitor	[72]
	Glycine hypocotyls	*Phytophthora*	[76]
Chalcone isomerase (CHI)	*Phaseolus* hypocotyls	*Colletotrichum*	[26]
	Phaseolus culture	Elicitor	[26]
Cinnamyl alcohol dehydrogenase	*Phaseolus* culture	Elicitor	[96]
	Other proteins and enzymes		
Casbene synthetase	*Ricinus* seedlings	Elicitors	[152]
Chitinase	*Phaseolus* leaves	*Pseudomonas*	[195]
PR proteins	*Nicotiana* leaves	TMV	[115, 116]
		Salicylic acid	[115, 116]
	Petroselinum culture	Elicitor	[186]
	Phaseolus leaves	AMV	[57]
Thaumatin-like protein	*Nicotiana* leaves	TMV	[44, 116]
		Salicylic acid	[44, 116]
HRGP	*Phaseolus* cultures	Elicitor	[57]
Proline hydroxylase	*Phaseolus* cultures	Elicitor	[27]

* Slusarenko, unpublished data.

[1] Data included in this table are for those defence-related genes for which increases in mRNA concentration or activity has been shown. Induced enzyme activity and *in vivo* labelling data are not included.

139, 144, 183, 184, 197]. Differential screening of cDNA libraries prepared from mRNA isolated at different times after inoculation can identify resistance-associated cDNA clones [44, 49, 51, 87, 98, 116, 159]. Although sequencing of the gene and computer searching of sequence banks might indicate a tentative function for the product of the gene in question [44], this approach must be coupled with biochemical and physiological investigations as hypotheses for possible functions emerge. The resistance-specific cDNAs can be used to probe genomic libraries of host DNA, thus enabling upstream regulatory sequences controlling gene expression to be studied.

Although the task is daunting, the shotgun approach has yielded valuable information on gene ex-

pression induced by heat shock [174, 175], anoxia [55, Dennis, pers. commun., 83, 170], wounding [178], fruit development [150, 180] and interactions of legumes with *Rhizobium* [22, 118, 194]. In one case this approach has led to the implication of a defence role for a previously undescribed protein [44].

We shall now consider in more detail some of the defence responses of plants to pathogens and elicitors where *de novo* gene expression has been demonstrated or is likely to be responsible for the effect.

Defence responses involving secondary plant metabolism

The majority of the responses under this heading involve phenylpropanoid metabolism. The biochemistry of phenolic substances, including phenylpropanoids, and their relevance in plant disease has been extensively reviewed elsewhere [61, 64, 84, 85, 86, 127, 138, 185]. The interrelationships between the biochemical pathways involved in phenylpropanoid metabolism are summarised in Fig. 2.

(1) Phytoalexins

Both the furanocoumarin class of phytoalexins from parsley (*Petroselinum crispum*) and the isoflavonoid phytoalexins of the Leguminosae are derived from phenylpropanoid precursors, and the targeted approach described above has yielded valuable information on the control of gene expression in response to pathogens. There is also evidence of gene expression induced in the production of casbene, the chemically distinct terpenoid phytoalexin of *Ricinis communis.*

(a) Parsley (Petroselinum crispum). Parsley cell suspension cultures, when treated with an elicitor preparation from a non-pathogen of parsley, *Phytophthora megasperma* f.sp. *glycinea*, showed increased mRNA activities for phenylalanine ammonia lyase (PAL) and 4-coumarate: CoA ligase (4CL), and accumulated antifungal furanocoumarins [105, 132]. In addition to PAL and 4CL, the mRNA activity for chalcone synthase (CHS) increased when cell suspension cultures were irradiated with UV [131]. Immunoprecipitation of PAL synthesised in *in vitro* translation reactions was used to measure the translatable activity of PAL mRNA [105]. As cDNA clones of PAL, 4CL and CHS became available, these were used to probe RNA dot blots to monitor changes in RNA concentration for those enzymes [131, 132]. Subsequently, run-off transcription experiments showed that increases in mRNA on UV light or elicitor stimulation of parsley cell suspension cultures was due to *de novo* transcription [38]. More recently it was reported that there were two 4CL genes and at least two, and possible three, different PAL genes in parsley; all the genes

Fig. 2. Interrelationship of different biosynthetic pathways of phenylpropanoid metabolism.

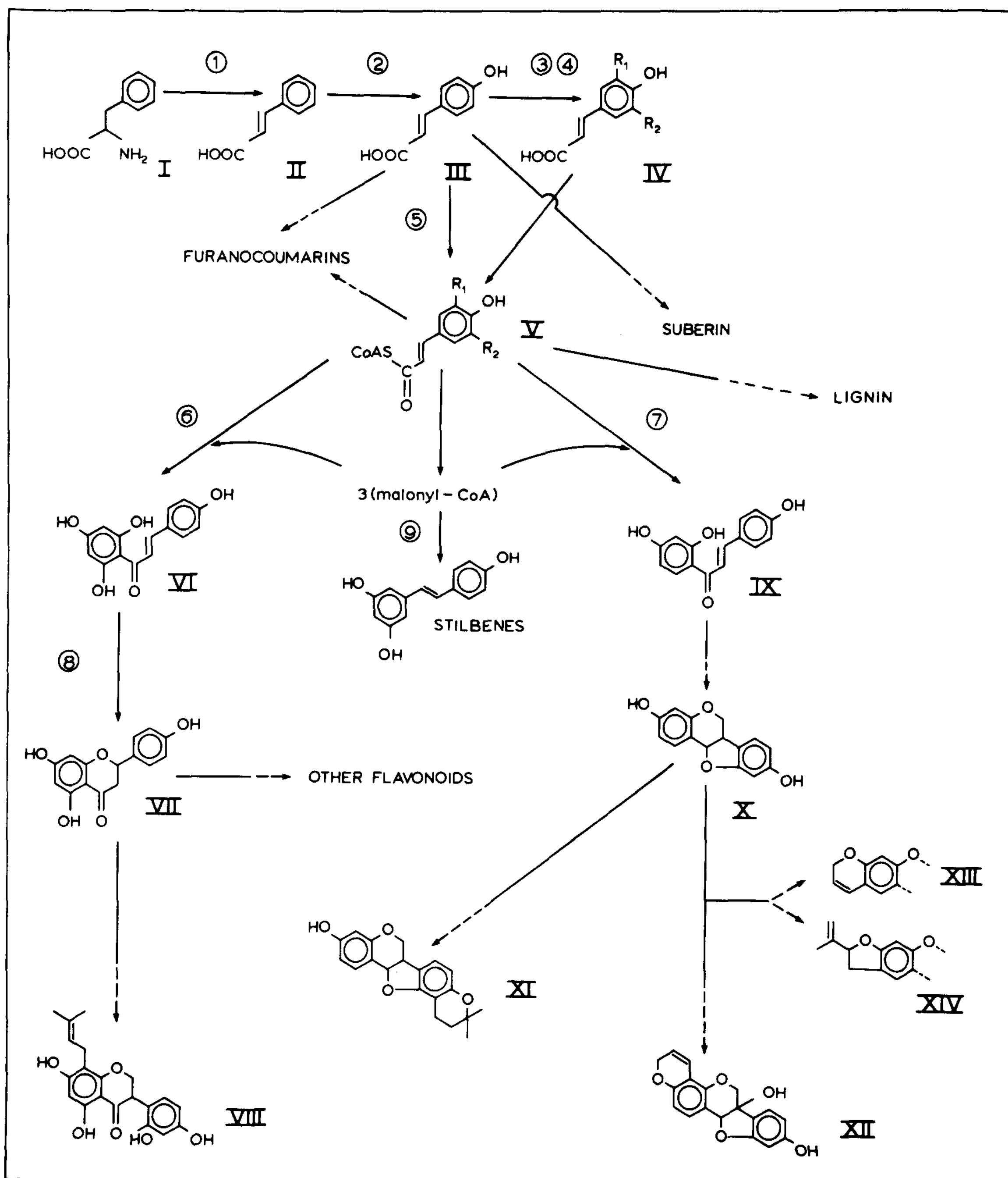
I	phenylalanine	X	3,9-dihydroxypterocarpan
II	*trans*-cinnamic acid	XI	phaseollin
III	*p*-coumaric acid (4-hydroxycinnamic acid)	XII, XIII, XIV	glyceollin 1,2,3
IV	$R_1 = OH$, $R_2 = H$: caffeic acid		
	$R_1 = CH_2O$, $R_2 = H$: ferulic acid		*Enzymes*
	$R_1 = CH_3O$, $R_2 = OH$: 5-hydroxyferulic acid	(1)	phenylalanine ammonia lyase (PAL)*
	$R_1 = R_2 = CH_3O$: sinapic acid	(2)	cinnamate-4-hydroxylase
V	cinnamyl CoA esters ($R_1 = R_2 = H$)	(3) & (4)	cinnamate-3 & 5-hydroxylase
	coumaroyl CoA	(5)	4-coumarate: CoA ligase (4CL)*
VI	2′,4,4′,6′-terahydroxychalcone	(6)	6′-hydroxychalcone synthase (CHS)*
VII	naringenin	(7)	6′-deoxychalcone synthase
VIII	kievitone	(8)	chalcone isomerase (CHI)*
IX	liquiritigenin	(9)	stilbene synthase

* Indicates that induction of the enzyme either in elicitation or challenge by a pathogen has been observed experimentally in at least one system (see text).

were expressed in response to elicitor and UV light [68, 102, 103, 129].

(b) French bean (Phaseolus vulgaris). Host gene expression in the phytoalexin response of French bean has been reviewed comprehensively by Dixon [61, 62, 63], and only the salient features will be covered here.

Using a range of techniques (Table 2), two model systems have provided a wealth of useful information. (a) Cell suspension cultures treated with elicitor derived from the bean pathogen *Colletotrichum lindemuthianum*. (b) Excised hypocotyls of bean inoculated with spore suspensions of the parent organism. The isoflavonoids phaseollin and kievitone



appear to be the most important phytoalexins which accumulate in French bean when it is challenged with fungal plant pathogens. Transcriptional activation of the genes encoding the phenylpropanoid biosynthetic enzymes, PAL and CHS, was observed within 5 minutes of elicitor treatment [133]. PAL and CHS appear to be coordinately regulated. However, chalcone isomerase (CHI) enzyme activity, rate of synthesis and mRNA activity may peak at later times than those of PAL and CHS under some circumstances [62, 65, 161]. The differences may depend upon the cultivar of French bean used since PAL, CHS and CHI were coordinately regulated in cell suspension cultures of cv. Canadian Wonder [45]. Certain elicitor preparations from *C. lindemuthianum* culture filtrates are able to induce CHS activity without inducing CHI [106]. CHI appears to be encoded by a single gene, whereas PAL and CHS are members of multigene families. There are at least 3 structural genes for PAL and 6 for CHS, and these appear to be differentially induced in resistance [61, 62].

(c) Soybean (Glycine max). The glyceollins 1, 2 and 3 (see Fig. 2) appear to be the most important phytoalexins which accumulate in soya bean when it is challenged with fungal plant pathogens, for example *Phytophthora megasperma* f.sp. *glycinea* [72, 73, 74]. *In vivo* labelling studies and *in vitro* translation of mRNA demonstrated that increases in the activity of PAL, 4CL and CHS were preceded by transient increases in their rates of synthesis [32, 72, 76, 91, 113, 173].

The activities of enzymes such as glucose-6-phosphate dehydrogenase and glutamate dehydrogenase which are not directly involved in glyceollin biosynthesis did not increase when intact roots of soya bean were inoculated with zoospores of *P. m.* f.sp. *glycinea* [30, 31]. In contrast, when hypocotyls were inoculated with mycelium of *P. m. glycinea*, marked increases in the activity of these enzymes were found irrespective of the race of *P. m. glycinea* used for inoculation [32]. This illustrates the importance of exercising caution in interpreting the results obtained when working with model systems.

(d) Castor bean (Ricinus communis). Casbene is a diterpenoid phytoalexin produced in *Ricinus communis* seedlings in response to pathogenic fungi including *Rhizopus stolonifer*. The activities of at least the last two enzymes involved in casbene synthesis increased following infection [69, 70, 71, 137]. Messenger RNA activity for the last enzyme, casbene synthetase, increased over six hours in *R. communis* seedlings following treatment with elicitor prepared from the fungus [152].

(2) Lignification

Lignin is a complex polymer, formed by the random condensation of phenylpropanoid units, and is an integral component of secondary cell walls of vascular plants. It is resistant to breakdown by many microorganisms [97, 104]. Enhanced cell wall lignifi-

Table 2. Techniques used to investigate the regulation of selected enzymes of phenylpropanoid metabolism in bean in response to elicitor or inoculation with *Colletotrichum lindemuthianum*

Technique	Enzyme			Ref.
	PAL	CHS	CHI	
In vivo density labelling gradient centrifugation	+		+	[64, 65]
In vivo pulse-labelling	+	+	+	[134]
In vitro translation followed by specific immunoprecipitation	+	+	+	[134]
Northern blots probed with cDNA	+	+		[75, 169]
Thiouridine labelling followed by organomercurial affinity chromatography and *in vitro* translation	+		+	[46]

cation has been observed in a number of plant species following challenge by various plant pathogenic fungi, viruses, nematodes and treatment with elicitors [19, 84, 191]. In *Raphanus japonica*, PAL and peroxidase activities and lignification were found to increase following infection with *Peronospora parasitica* and *Alternaria japonica* [7, 8, 9, 10, 11, 12]. Enhanced lignification and PAL activity were recorded from resistant but not susceptible discs of different potato varieties following challenge by *Phytophthora infestans* [111]. Ferulate and particularly *p*-coumarate: CoA ligase activities increased in cell walls of *Cucumis melo* following infection by *Colletotrichum lagenarium*, and lignification was found to be associated with resistance to the pathogen [95]. Lignification has also been found in *Cucumis sativa* following treatment with oligogalacturonides and polygalacturonate lyase from *Cladosporium cucumerinum* [162]. Resistant melon varieties were found to produce more hydroxyproline-rich glycoprotein (HRGP, see below) and lignin than susceptible varieties, and to do so more rapidly [107]. Cinnamyl alcohol dehydrogenase (CAD) activity (the first committed enzyme of lignification), and mRNA activity increased in *Phaseolus vulgaris* cell suspension cultures following treatment with elicitor from *C. lindemuthianum* [96]. A cDNA clone for the CAD gene of *P. vulgaris* has been obtained recently. RNA hybridisation analysis using this clone demonstrated that lignification was induced concomitantly with phytoalexin production in this system [96].

Lignification appears to be a major induced structural defence mechanism in grasses. The measurable activities of several enzymes associated with lignification increased in leaf discs of the grass *Phalaris arundinacea* following challenge with the non-host pathogenic fungus *Helminthosporium avenae* [192]. In this interaction low pI (cathodic) but not high pI (anodic) peroxidase isozymes were induced [190]. Similarly, increases in peroxidase and polyphenol oxidase activities were associated with resistance in mung bean (*Vigna radiata*) induced by *Rhizoctonia solani* and ethephon (which releases ethylene) [6]. It is not clear whether these increased enzyme activities are associated with lignification in this system or the deposition of other phenolics. PAL and tyrosine ammonia lyase (TAL) activities (shown in this species to be the same enzyme), 4CL, 4-cinnamate hydroxylase, caffeic acid *o*-methyl transferase and 5-hydroxyferulic acid *o*-methyl transferase were induced in wheat (*Triticum aestivum*) by *Botrytis cinerea* [148, 149]. Lignification was associated with HR in wheat cultivars resistant to *Puccinia graminis* f.sp. *tritici* [18].

Plant cell wall modification other than lignification

The structure of plant cell walls was recently reviewed by Fry [88]. Extensin, a hydroxyproline-rich glycoprotein (HRGP), plays a major structural role in the cell wall, probably forming an interlinked net complementing the cellulose mesh [202]. There have been several reports of increases in HRGP in wounded or pathogen-inoculated tissues of French bean (*Phaseolus vulgaris*), cucumber (*Cucumis sativus*), melon (*Cucumis melo*), potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and rice (*Oryza sativa*) [151]. Two HRGPs accumulated in wounded carrot (*Daucus carota*) tissue, one of which was shown to be extensin [39, 40]. The content of HRGP (possibly extensin) in melon seedlings underwent a tenfold increase following infection with *Colletotrichum lagenarium* [78]. This increase correlated with increased resistance to the pathogen, was mediated by ethylene, was not attributable to wounding and was reversible when HRGP biosynthesis was suppressed by treatment with free hydroxyproline [77, 79, 189]. HRGP and ethylene production were also induced in melon by the addition of elicitor prepared from *C. lagenarium* cell walls, and from melon tissue [165, 188]. HRGP induction has also been recorded from soya bean (*Glycine max*) treated with elicitor from *Phytophthora megasperma* [165], and in *Phaseolus vulgaris* cell culture following treatment with elicitor from *C. lindemuthianum* [28, 29]. Messenger RNA for HRGP accumulated in cell cultures following treatment with elicitor and in hypocotyls in race-cultivar interactions [179]. The accumulation of HRGP in *P. vulgaris* cell culture was associated with increases in the

activities of the enzymes, proline 2-oxoglutarate dioxygenase (prolyl hydroxylase) and a protein arabinosyl transferase [28, 29]. There is recent evidence that mRNA activity for the prolyl hydroxylase associated with HRGP accumulation also increases following elicitor treatment [27].

Callose, a β-1,3-glucan, accumulates in plant cell walls in response to physical and chemical stress and is a major component of papillae or wall appositions formed at sites of attempted penetration by invading fungal hyphae [2, 19]. Callose surrounds local lesions in some viral infections where it may help to prevent spread of the virus [19, 176]. However, callose synthesis may be mediated by an influx of Ca^{2+} ions into the cells which directly activates the β-1,3-glucan synthase enzyme which is localised in the plasmalemma, rather than by *de novo* gene expression [128].

Hydrolytic enzymes

(1) Chitinase

Endochitinase activity increases in plants after inoculation with fungal, bacterial and viral plant pathogens [147, 156, Meins, pers. commun.] and after treatment with ethylene or elicitors [25, 147, 166]. Many plants treated with elicitors or inoculated with pathogens produce both ethylene and chitinase. Ethylene may act as a second messenger for chitinase induction [166]. Indeed, in melon seedlings (*Cucumis melo*) treated with elicitor from *Colletotrichum lagenarium*, inhibition of ethylene biosynthesis by treatment with aminoethoxyvinylglycine (AVG) also inhibited chitinase induction [166]. However, in pea plants (*Pisum sativum*) inoculated with *Fusarium solani* f.sp. *phaseoli* or treated with elicitors, ethylene biosynthesis could be suppressed without inhibiting the induction of chitinase [147]. Thus, different control mechanisms appear to exist for the induction of chitinase in different plant species. In French bean (*Phaseolus vulgaris*), chitinase is encoded by a multigene family and at least two of these genes are expressed in response to ethylene [34]. Chitin, the substrate for chitinase, does not occur commonly in plants, although it is a common constituent of many fungal cell walls. Both chitin and chitosan (deacetylated chitin) can serve as elicitors of plant defence reactions in their own right [99]. Plant chitinases are potent inhibitors of fungal growth [172]. Endochitinase also shows lysozyme activity and its induction may therefore be an effective defence mechanism of plants against invading bacteria [24, 25].

In the interaction between French bean (*Phaseolus vulgaris*) and *Pseudomonas syringae* pv. *phaseolicola* chitinase mRNA activity was monitored by immunoprecipitation of *in vitro* translation products with antiserum raised against bean chitinase [195]. Chitinase mRNA was detected as early as six hours after inoculation of leaves with an avirulent isolate of *P. s.* pv. *phaseolicola*. In contrast, activity was detected first at 24 hours after inoculation with a virulent isolate. These results suggest an early, specific induction of the chitinase gene, or genes, in the incompatible combination.

(2) β-1,3-glucanase

The increase in β-1,3-glucanase activity following ethylene treatment of bean tissue has been known for some time [1]. β-1,3-glucanase activity increases following elicitor treatment of parsley (*Petroselinum crispum* (*P. hortense*) cells [130]. β-1,3-glucanase can act to release elicitor-active carbohydrate fractions from β-1,3-glucans of fungal cell walls [124, 125]. Both β-1,3-glucanase and chitinase are induced in *Fusarium*-inoculated pea (*Pisum sativum*) [153]. Activities of other polysaccharide-degrading enzymes tested did not increase [153].

Pathogenesis-related proteins

Pathogenesis-related proteins (PR proteins or B proteins) are induced in a number of plant species following infection [193]. They have been detected in leaves infected with plant pathogenic bacteria, fungi and viruses and abiotic elicitors, UV light and ethephon. They have also been found in tobacco (*Nicotiana tabacum*) at lower concentrations in distant, uninfected leaves following infection with tobacco

mosaic virus (TMV). Levels in tobacco are highest in the margins of hypersensitive lesions [5], and they are produced earlier in tomato (*Lycopersicon esculentum*) showing an incompatible reaction to *Fulvia fulva (Cladosporium fulvum)* than in a compatible reaction [60]. Their appearance also correlates with leaf senescence and stress, and they are found in tobacco callus, as well as in leaves following infection with pathogens. Where characterised, they have the common properties of being acid-extractable, protease-resistant, of extracellular location and of relatively low M_r. Those of tobacco *(Nicotiana tabacum)*, parsley *(Petroselinum crispum)* and bean (*Phaseolus vulgaris*) have low isoelectric points [193]; that of tomato (*Lycopersicon esculentum*) has a high isoelectric point [36]. Electrophoretic analyses of tobacco proteins labelled with ^{14}C-labelled amino acids indicated that at least ten PR proteins are associated with HR to TMV [117]. Genes for the tobacco [115, 116] and parsley [186] PR proteins have recently been cloned following the demonstration of *de novo* induction of PR mRNA by TMV in two cultivars of tobacco [37, 115], and in elicitor-treated parsley cells [186]. Messenger RNA concentrations for four PR proteins increase in *Phaseolus vulgaris* following treatment with $HgCl_2$ or alfalfa mosaic virus [56, 57]. Comparisons of their sequences with the gene sequence data banks indicates that they do not fall into any previously known functional class of protein and show considerable sequence diversity within the group [116, 140]. Their role in disease resistance is supported by the observed acquired resistance to TMV following treatment with salicylic acid, an effective inducer of PR protein production, which also acts to protect tobacco from subsequent infection by TMV [116, 200]. Despite these indications, their roles and functions in stress and disease resistance remain unknown [193].

Proteinase (protease) inhibitors

Polypeptide inhibitors of protease are widely distributed in all plant tissues and are thought to have a role in defence against herbivores since they tend to be active against animal and not endogenous proteases [168]. Two wound-induced protease inhibitors occur in tomato (*Lycopersicon esculentum*), and can account for up to 10% of the soluble protein 48 hours after severe wounding. They are induced in leaves remote from the injury following the production of an oligo-galacturonide inducer [92, 93, 94, 136, 167, 198]. A wound-induced proteinase inhibitor which shows considerable sequence homology to the tomato protein has been isolated from wounded potato (*Solanum tuberosum*) tubers [171]. The levels of protease inhibitors increase following treatment of melon with elicitor prepared from *Colletotrichum lagenarium* [79].

Other proteins

Interaction-specific glycosidases and peroxidases were detected in intercellular fluids of wheat (*Triticum aestivum*) infected with *Puccinia graminis* f.sp. *tritici*, but it is not certain whether these were of host or pathogen origin [114]. A number of enzymes involved in primary and secondary metabolism were tested for induction following treatment of *Phaseolus vulgaris* cell suspension cultured with elicitor, and *Pisum sativum* inoculated with *Fusarium solani* f.sp. *phaseoli*. In bean, only those enzymes involved in phenylpropanoid metabolism were induced [160], whereas in pea, of the hydrolytic enzymes tested, only chitinase and β-1,3-glucanase activities increased [152]. RNA-dependent RNA polymerases may play a role in interactions of viruses with some plants [82].

Several proteins have been shown to decrease in quantity in infected tissues. mRNA for both the small and large subunits of ribulose bisphosphate carboxylase (rubisco) decreases in a co-ordinated manner following *Colletotrichum lagenarium* infection of melon (*Cucumis melo*, [164]).

The hypersensitive reaction (HR)

The hypersensitive reaction can occur when plants are challenged by viruses, fungi, bacteria or nematodes. When it occurs, it is invariably associated with resistance (although some viruses can escape the

hypersensitive lesion [90]), yet its role in the resistance response is not at all clear [108]. The dead cells observed at the end of the HR are simply an indicator that HR has occurred and it is important to include all the facets of HR development in any consideration of this response [126]. Thus, one must consider recognition of the pathogen by the plant, induction of the reaction, development of the necrosis, formation of antimicrobial substances and localisation of the pathogen. Bailey [15] observed that host cell injury, often leading to cell-death, was a common denominator in the action of biotic and abiotic elicitors and pathogen challenge which preceded the accumulation of phytoalexins. He postulated that injured cells released constitutive elicitors into the surrounding healthy cells which responded by synthesising phytoalexins. In the compatible interaction, the rapid accumulation of phytoalexins was not observed in an initial biotrophic phase where gross cell injury was avoided. This attractive hypothesis could explain the specificity observed in the interactions between differential cultivars and physiological races of the pathogen in terms of lack of recognition and HR induction in the compatible combination. Moreover, hypersensitive cell death appears to require a period of host protein synthesis before it can occur [121]. This led to speculation that HR was a kind of programmed cell death which might regulate the expression of genes involved in resistance [181]. Thus, it was postulated that the early events in HR were the most significant, while the endpoint of necrosis was an incidental outcome of those early reactions. In other words, necrosis is a visible marker that HR had occurred. Indeed, induction of defence gene transcripts in bean (*Phaseolus vulgaris*) hypocotyls inoculated with spores of *Colletotrichum lindemuthianum* is extremely rapid and precedes visible HR flecking [133].

Early changes (from 2 h) in gene expression in intact leaves of French bean (*Phaseolus vulgaris*) undergoing HR to *Pseudomonas syringae* pv. *phaseolicola* have been documented [182, 183]. Necrosis occurs in this system from 21 to 25 h after inoculation with an avirulent isolate, and probing of RNA blots with a cDNA clone for bean PAL showed that PAL mRNA began to increase between 6 and 9 h after inoculation and peaked around 12 h. Since PAL is the key early enzyme in phenylpropanoid metabolism and is induced in phytoalexin biosynthesis (see section above), these results showed clearly that several changes in gene expression precede both phytoalexin biosynthesis and necrosis in intact plant tissue inoculated with cells of an avirulent bacterial pathogen.

Messenger RNA prepared from *Brassica campestris* leaves developing HR to *Xanthomonas campestris* pv. *vitians* encodes novel, and as yet unidentified, polypeptides as early as 4 h following inoculation [41]. Visible HR has not been detected in this system before at least 8 h post inoculation.

However, there is no direct evidence that any of these polypeptides have a role in HR.

Figure 3 shows a schematic representation of the possible interrelationships between the HR and other plant defence responses. There appear to be fundamental differences in the elicitation of HR and the associated phytoalexin accumulation in plants by bacteria and fungi (in those species shown to accumulate phytoalexins). Heat-killed [141, 142] or UV-killed (Slusarenko, unpublished) cells of *Pseudomonas syringae* pv. *phaseolicola* neither induce HR, nor cause accumulation of phytoalexins. Similarly, heat-killed and antibiotic-treated cells of *X. c. vitians* do not induce HR in *B. campestris* leaves [41]. Living bacterial cells are apparently required for induction of HR and accumulation of phytoalexins. In contrast to the situation with fungi [58], there are few examples of elicitors derived from bacterial sources: e.g. pectic enzymes produced by *Erwinia* spp. kill plant cells and elicit phytoalexin accumulation [143, 14], and glycoproteins solubilised from the cell walls of *P. s. glycinea* elicit phytoalexin accumulation with the same specificity as the parental races of the bacteria [35]. It should be emphasised that elicitors are substances which visually mimic the effects of avirulent pathogens, and do not necessarily induce all the features of HR. Although the mechanisms are unclear, we believe that HR may play an important role in determining the outcome of natural infections of plants by pathogenic microorganisms.

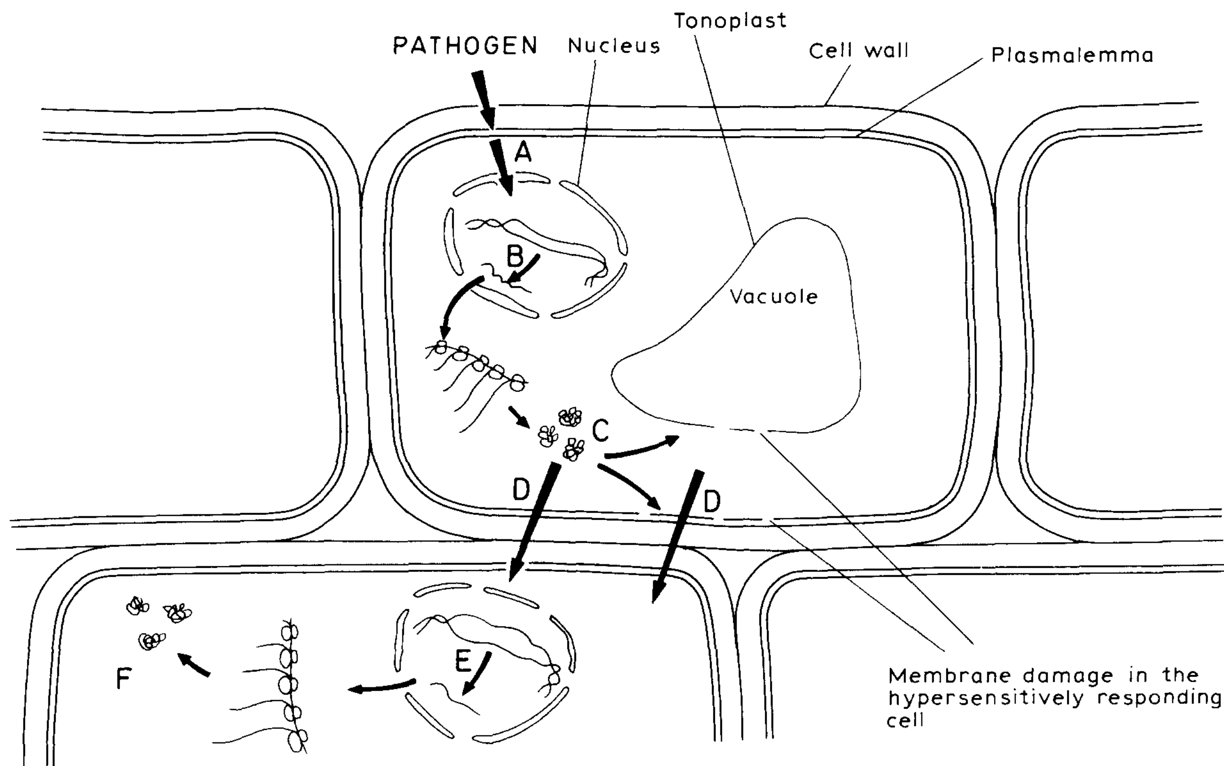


Fig. 3. Schematic representation of the possible interrelationships of the hypersensitive reaction (HR) to other plant defence responses.

A Signal transmission from the avirulent pathogen to the host cell nucleus, either directly or indirectly via second messengers.
B Primary response, i.e. transcription of genes whose products cause HR cell collapse and transcription of genes for defence responses independent of the HR.
C Gene products for HR independent responses and gene products which bring about membrane damage and lead to cell collapse.
D Intercellular signalling.
E Induction of defence gene transcripts in surrounding cells. This step may by-pass B and C (see text).

Conclusions

In this review we have catalogued evidence that a number of genes are selectively expressed in plants following challenge by bacterial, fungal and viral pathogens. The identity of these genes has been determined in a number of cases (Table 1), and it is clear that, at least in pea (*Pisum sativum*) and French bean (*Phaseolus vulgaris*), some of the same host genes are expressed in response to both fungal and bacterial pathogens [21, 49, 98, Slusarenko, unpublished]. It is also apparent that some changes in the expression of genes involved in defence reactions also occur, albeit to a later or lesser extent, during the development of disease in the same host. Furhermore, in those few examples studied in sufficient detail, some of the genes are also expressed in response to other stimuli, for example, wounding, treatment with abiotic elicitors, and UV light (see section on phytoalexins, above) [170]. The induction of a number of genes, each by several stimuli, does not imply that a single regulatory mechanism is involved, and the main research effort in molecular plant pathology over the next few years will be directed towards elucidating the mechanisms by which defence gene expression is regulated and coordinated.

It is not clear why some pathogens are recognised as being a potential threat to the plant and are met with a resistance response, whereas other, often closely related, forms are able to cause disease. Many

models for the triggering of the resistance response invoke the interaction of pathogen-derived products with host-derived receptors encoded by resistance genes [48, 54, 123, 145]. It should be noted that the study of pathogen-induced plant gene expression is unlikely to permit direct identification of such recognition/resistance genes since, by their nature, these should be constitutively expressed. The link between recognition/resistance genes and the expression of the defence response genes described in this review could be through passive depolarisation of membranes [13, 155, 157], or signal molecules such as Ca^{2+}, cAMP or inositol triphosphate. The potential role of these substances to act as second messengers in triggering plant defence responses has been discussed elsewhere [62, 73, 119]. If, however, the link between the recognition event and the expression of the genes for the defence responses are induced polypeptides, then it ought to be possible to identify them through the use of the shotgun method described above. The rapidity of transcriptional activation of PAL and CHS genes in bean cell cultures (within five minutes of treatment with elicitor) [133] argues against the induction of signal polypeptides in this system. An interesting factor is that, at least in some cases, there is more than one structural gene for the proteins concerned with the defence response. For example, at least four different isozymes of the enzyme PAL occur in French bean. These vary in K_m and pI, and show differential regulation; two isozymes are differentially induced by elicitor treatment [26]. Multigene families for both PAL (comprising three members) and CHS (with six members) have recently been identified [61, 62], but it is not yet clear how these structural genes relate to the different isozyme forms of the enzymes, or how their expression is regulated.

Identification of resistance-specific clones in the shotgun approach (see above) by differential hybridisation [87] yields clones which reflect large changes in relatively high-abundance mRNAs, but changes in low-abundance messages and small changes in abundant mRNAs are easily missed using this technique [23, 146, 201]. Thus, much of the fine-tuning of gene expression in resistance responses cannot be approached easily by this means. Differential screening can be made more efficient by pre-hybridisation of common mRNA sequences and separation of double- and single-stranded molecules by hydroxylapetite chromatography or by the so-called "sandwich" hybridisation method [23]. By these means, enriched single-stranded cDNA probes for induced mRNA species can be prepared for screening cDNA libraries. Hybrid-selected translation offers a more sensitive method than differential hybridisation to detect changes in low-abundance mRNAs in complex mixtures [201]. However, as a screening technique for the large number of clones present in cDNA libraries, hybrid-selected translation is a very labour-intensive undertaking.

Finally, we would like to comment on the future study of pathogen-induced plant gene expression. Application of existing techniques of molecular and cell biology is clearly going to give a fuller picture of the nature of this gene expression, by identifying more of the genes involved and determining the control of expression of such genes in any given plant species in response to different pathogens. There is already some progress towards determining the distribution of gene expression in different cell types in infected or challenged tissue [21]. Clearly there is plenty of scope for the extension of such studies using the genes already cloned for *in situ* hybridisations. Similarly, immunohistological techniques may be applied to study the distribution of polypeptide products of the genes involved [33, 163, 199]. These techniques should determine the partitioning of gene expression in the hypersensitively dying cells and adjacent, healthy cells. More refined studies using cell cultures with a combination of fractionated elicitors, elicitors from different kinds of pathogen and probes specific to unique sequences in the structural genes for different isozymes of a particular enzyme, should allow dissection of the diverse mechanisms of regulation of gene expression [52, 106]. Since a number of cloned genes are now available it will be possible to look at factors which directly trigger their expression in plants exhibiting resistance responses. Recent advances in the techniques of plant transformation and genetic manipulation hold the promise that transgenic plants containing cloned defence-related genes will soon be constructed and the regulation of these genes can be tested more clearly. These problems can be approached by using

promotor deletion analyses to detect transient expression in transformed plant cells or following stable incorporation into transgenic plants.

Induction of defence gene transcripts appears to be a cascade-type response and gel retardation assays to highlight DNA-binding proteins might be used successfully to identify trans-acting regulatory elements [176]. For example, transcriptional activity can be monitored by DNAse hypersensitivity mapping, and DNA "footprinting" techniques can be used to identify the attachment sites of DNA-binding proteins and hence determine the relevant regulatory sequences [196, 203]. Where multigene families are involved (e.g. PAL and CHS in French bean, see above) the application of such techniques will be particularly fruitful since individual members are differentially expressed in resistance responses.

In most cases, there is no direct evidence that the pathogen-induced responses observed constitute effective defence mechanisms. In many cases there is a wealth of circumstantial evidence that this is the case: for example, both phytoalexins and chitinase can inhibit fungal growth *in vitro* [17, 172]. This does not necessarily mean that these factors are effective against fungi or other pathogens *in vivo*. The combination of site-directed mutagenesis and transformation of host cells may facilitate the assignment of a direct role for any given gene in resistance.

Thus, the next few years look very promising indeed and will no doubt result in a much clearer understanding of the molecular biology of host-pathogen interactions. This may ultimately lead to one of the major goals for plant genetic engineering in agriculture, namely the development of disease-resistant plants.

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