Differential Accumulation of Plant Defense Gene Transcripts in a Compatible and an Incompatible Plant-Pathogen Interaction

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Phenylalanine ammonia-lyase and chalcone synthase catalyze the first reaction of phenylpropanoid biosynthesis and the first reaction of a branch pathway specific for flavonoid-isoflavonoid biosynthesis, respectively. These enzymes are key control elements in the synthesis of kievitone, phaseollin, and related isoflavonoid-derived phytoalexins. RNA blot hybridization with ³²P-labeled cDNA sequences was used to demonstrate marked accumulation of phenylalanine ammonia-lyase and chalcone synthase mRNAs in excision-wounded hypocotyls of *Phaseolus vulgaris* L. (dwarf French bean) and during race-cultivar-specific interactions between hypocotyls of *P. vulgaris* and the partially biotrophic fungus *Colletotrichum lindemuthianum*, the causal agent of anthracnose. In an incompatible interaction (host resistant), early concomitant accumulation of phenylalanine ammonia-lyase and chalcone synthase mRNAs, localized mainly but not entirely in tissue adjacent to the site of infection, was observed prior to the onset of phytoalexin accumulation and expression of localized, hypersensitive resistance. In contrast, in a compatible interaction (host susceptible) there was no early accumulation of these transcripts; instead, there was a delayed widespread response associated with phytoalexin accumulation during attempted lesion limitation. Two-dimensional gel electrophoresis of [³⁵S]methionine-labeled polypeptides synthesized in vitro by translation of isolated polysomal RNA demonstrated stimulation of the synthesis of characteristic sets of phenylalanine ammonia-lyase and chalcone synthase isopolypeptides in directly infected tissue and distant, hitherto uninfected tissue in both compatible and incompatible interactions. Our data show that specific accumulation of plant defense gene transcripts is a key early component in the sequence of events leading to expression of defense responses in wounded tissue and in infected tissue during race-cultivar-specific interactions and that an elicitation signal is transmitted intercellularly in response to infection.

Plants exhibit natural resistance to disease, which involves inducible defense mechanisms, including accumulation of phytoalexins, deposition of ligninlike material, accumulation of cell wall hydroxyproline-rich glycoproteins, and increases in the activity of certain hydrolytic enzymes, such as chitinase and glucanase (38). Such responses can be induced not only by infection but also by glycan, glycoprotein, and lipid elicitors which have been isolated from fungal cell walls and culture filtrates and, in some cases, by structurally unrelated artificial elicitors or mechanical damage (10, 14).

The molecular mechanisms underlying the operation of plant defense responses have been most intensively studied in relation to the stimulation of phytoalexin biosynthesis in elicitor-treated cell cultures (10, 14). Thus, in cell suspension cultures of *Phaseolus vulgaris* L. (dwarf French bean) elicitor treatment causes marked but transient increases in the rate of synthesis of phenylalanine ammonia-lyase (PAL), the first enzyme in general phenylpropanoid metabolism, and in the rates of synthesis of chalcone synthase (CHS) and chalcone isomerase, the first two enzymes specific to the flavonoid-isoflavonoid branch pathway of phenylpropanoid biosynthesis, concomitant with the onset of accumulation of kievitone, phaseollin, and related isoflavonoid phytoalexins (8, 13, 27, 28). Increases in the rates of enzyme synthesis can be observed within 20 min after elicitor treatment, with maximum rates occurring 3 to 4 h after elicitation (8, 28). The transient increases in enzyme synthesis rates reflect in-

creases in the levels of the corresponding mRNA activities (8, 29), which, together with changes in the apparent stabilities of the enzymes in vivo (16, 30), are responsible for the marked increases in enzyme activity that control phytoalexin accumulation in response to elicitors.

When cloned PAL and CHS cDNA sequences are used as probes in RNA blot hybridizations, a close correspondence between induction of hybridizable mRNA and increased mRNA activity is observed (17, 36). The rapid accumulation of PAL and CHS mRNAs from very low, almost undetectable basal levels in unelicited cells strongly suggests that the elicitor stimulates the transcription of PAL and CHS genes. Transcriptional activation of PAL and CHS genes and induction of mRNA synthesis following elicitor treatment of suspension-cultured cells have been confirmed by in vivo labeling of newly synthesized RNA with 4-thiouridine (9) and by in vitro labeling of runoff transcripts in isolated nuclei (unpublished data). These and similar observations in other systems (7, 26) indicate that the elicitor causes a massive change in the overall pattern of RNA synthesis, leading to induction of mRNAs which encode enzymes of phytoalexin biosynthesis and other defense proteins (9, 36; C. J. Lamb, J. N. Bell, C. L. Cramer, S. L. Dildine, C. Grand, S. A. Hedrick, T. B. Ryder, and A. M. Showalter, in J. B. St. John, ed., *Biotechnology for Solving Agricultural Problems*, in press).

Although elicitor treatment of cell cultures represents a system of reduced complexity which greatly facilitates molecular analysis, a major question is whether similar mechanisms operate during attempted infection of intact plant

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FIG. 1. Dissection of hypocotyl tissue.

tissue, particularly during genetically controlled race-cultivar-specific interactions, which underlie many agriculturally important plant diseases (12). Furthermore, since intercellular transmission of elicitation (15) and other stress signals (35) may operate differently in intact tissue than in isolated cells, it is also important to determine the spatial pattern of the response of intact plant tissue to microbial attack under conditions closely resembling the natural infection process.

Therefore, we initiated a study of phytoalexin gene expression in race-cultivar-specific interactions between hypocotyls of *P. vulgaris* and the partially biotrophic fungus *Colletotrichum lindemuthianum*, the causal agent of anthracnose (1–3). Hypocotyls, a natural site of the disease, can be reproducibly infected, without prior mechanical damage, by surface inoculation with conidia, the natural infective propagules. Detailed physiological studies have established the biological importance of phytoalexin accumulation in this system, which, therefore, provides a model for biochemical analysis of disease resistance under conditions closely resembling the natural infection process (1–3). Previous studies have shown that infection causes a marked stimulation of the synthesis of phytoalexin-biosynthetic enzymes, as judged by *in vitro* translation of isolated polysomal RNA (4, 8). In this study we used RNA blot hybridization with ^{32}P -labeled PAL and CHS cDNA sequences to demonstrate clear temporal and spatial differences between a compatible interaction and an incompatible interaction in the pattern of accumulation of PAL and CHS transcripts. Rapid marked accumulation of these transcripts was also observed in excised hypocotyl tissue. Our data suggest that specific activation of plant defense genes is a key early component in the expression of defense responses both in wounded tissue and in infected tissue during race-cultivar-specific interactions and that an elicitation signal is transmitted intercellularly in response to infection.

MATERIALS AND METHODS

Plant and fungal material. Seeds of *P. vulgaris* L. cv. Kievitsboon Koekoek were germinated as previously described and were grown at 25°C and 85% relative humidity under fluorescent light with a 16-h photoperiod (3). After 7 days, fully extended hypocotyls were excised 10 mm from the base and 20 mm below the cotyledons. The ends were sealed with molten paraffin wax, and the hypocotyls were incubated horizontally in humidified boxes at 16°C under the same light conditions.

C. lindemuthianum races β and γ were maintained and sporulating cultures were generated by UV irradiation as previously described (3). Spores obtained after 6 to 8 days were suspended in distilled water, washed twice by centrifugation at $800 \times g_{av}$ for 3 min, and finally suspended in

distilled water at a concentration of 5×10^5 spores per ml. Drops (5 μl) of this suspension were applied at 20-mm intervals along the upper surfaces of the hypocotyls. Lesion development was monitored visually and microscopically. Control hypocotyls were treated with sterile water in the absence of spores and incubated separately as described above. At appropriate intervals, 40 to 50 infected hypocotyls were collected, the terminal 5-mm portions were removed, and the remaining material was excised with a scalpel to yield collections of tissue from different regions of the hypocotyl in relation to the initial sites of spore inoculation (Fig. 1). Thus, the hypocotyls were bisected longitudinally in a horizontal plane, and the upper one-half was then divided into two series of equal portions comprising the tissue immediately underlying the initial sites of inoculation (site 1) and the residual portion of tissue between sites of inoculation (site 2). The lower one-half of the hypocotyls was designated site 3, and the control hypocotyls after removal of the wounded terminal portions were designated site 4. Typically, at each time point between 150 and 210 samples of each type of site were collected, giving 4 g (fresh weight) of site 1 and site 2 material and 7 g (fresh weight) of site 3 and site 4 material. Harvested tissue was frozen in liquid N_2 and stored at -70°C until it was required for mRNA preparation. For wound induction, hypocotyl segments (5 mm) were excised from 9-day-old seedlings and incubated in 5 mM potassium phosphate buffer (pH 5.5) at 25°C under sterile conditions as previously described (39).

Isolation of RNA. Polysomal RNA was isolated by a modification (37) of the method of Palmiter (33). Total cellular RNA was isolated from tissue that was homogenized directly in a phenol–0.1 M Tris (pH 9.0) emulsion (20). The method used for further purification of the phenol-extracted total RNA was identical to that used for polysomal RNA. Extraction of RNA from infected hypocotyls of *P. vulgaris* has been described previously (4). RNA was assayed spectrophotometrically at 260 nm.

RNA blot hybridization. RNA (5 μg) was denatured with glyoxal, separated by electrophoresis on a 1.2% agarose gel in 10 mM phosphate buffer (pH 7.0) (31), blotted onto nitrocellulose (41), and hybridized with ^{32}P -labeled *P. vulgaris* PAL and CHS cDNA sequences prepared by nick translation of plasmids pPAL5 and pCHS5, respectively, as previously described (17, 36). Following autoradiography, PAL and CHS mRNAs were quantitated by scanning densitometry calibrated with reference to a set of internal standard mRNA samples comprising appropriate dilutions of an mRNA preparation from elicitor-induced cells. Several autoradiograms, exposed for different periods, were obtained from each blot to enable quantitation of each sample in the linear range of film response. The time course of mRNA accumulation in each tissue site was monitored directly within a single blot. The relative accumulation

compared with other tissue sites was calibrated by referring to the internal standards and was further checked by hybridization within the same blot of maximally induced mRNA samples from each set of tissue sites.

In vitro translation and two-dimensional gel electrophoresis. Isolated polysomal RNA was translated in vitro in the presence of [35 S]methionine (Amersham Corp.) by using a message-dependent reticulocyte lysate (34), as previously described (29). In vitro translation products were fractionated by two-dimensional gel electrophoresis, using the method of Garrels (19) and a pH range of 3.5 to 10 for isoelectric focusing (first dimension), followed by sodium dodecyl sulfate–10.0% polyacrylamide gel electrophoresis (second dimension) and fluorography (6). PAL and CHS subunits were immunoprecipitated indirectly by using protein A-Sepharose in conjunction with monospecific antisera (5, 29). For two-dimensional analysis, immunoprecipitates were dissolved in 20 μ l of 4.2% (wt/vol) sodium dodecyl sulfate containing 10% (vol/vol) 2-mercaptoethanol and boiled for 3 min. A 5- μ l sample was then mixed with 5 μ l of 0.48 M Tris hydrochloride (pH 7.0) containing 0.5 mg of RNase (Worthington Diagnostics) per ml, 1.0 mg of DNase (Worthington) per ml, and 0.05 mol of $MgCl_2$ per liter and incubated at 0°C for 5 min (5). After nuclease treatment the solution was incubated at 20°C for 10 min with 25 μ l of a solution containing 9.95 mol of urea (Schwarz/Mann) per liter, 4% Nonidet P-40 (Bethesda Research Laboratories, Inc.), 2% ampholines (pH 3.5 to 10) (LKB Instruments, Inc.), and 0.1 mol of dithiothreitol (Calbiochem-Behring) per liter.

RESULTS

RNA was isolated from tissue directly underlying the site of spore inoculation (site 1), from tissue laterally adjacent to the infected tissue (site 2), from tissue beneath sites 1 and 2 (site 3) as shown in Fig. 1, and from equivalent control uninoculated hypocotyls (site 4). The changes in the levels of PAL and CHS mRNAs were measured by RNA blot hybridization with 32 P-labeled *P. vulgaris* cDNA sequences. The PAL and CHS cDNAs specifically hybridized with mRNA species which were about 2.5 and 1.4 kilobases long, respectively, and were present in polysomal and total cellular RNA samples isolated from infected hypocotyls (Fig. 2). These transcripts were the same size as previously established for PAL and CHS mRNAs in elicitor-treated cell cultures (17, 36).

Following application of spores of *C. lindemuthianum* to the unwounded surfaces of hypocotyls of *P. vulgaris* cv. Kievitsboon Koekoek, there is a period of 30 to 40 h during which the spores germinate and the fungus penetrates the cuticle (1–3, 32). In an incompatible interaction with race β we observed an early, transient, concomitant accumulation of hybridizable PAL and CHS mRNAs in the total cellular RNA samples isolated from tissue immediately adjacent to the site of spore inoculation (site 1). Marked accumulation was observed at 52 h, with maximum levels occurring at 69 h after spore inoculation such that at the onset of phytoalexin accumulation and expression of hypersensitive resistance the levels of hybridizable PAL and CHS mRNAs were between 80- and 100-fold above their respective levels in equivalent, uninfected control hypocotyls (Fig. 3 and 4). A detailed analysis of the early stages of the incompatible interaction with race β revealed that accumulation of hybridizable PAL and CHS mRNAs in the total cellular RNA fraction could be observed as early as 39 h after spore inoculation (Fig. 5) (i.e., about 30 h before the onset of

FIG. 2. Blot hybridization of RNAs isolated from infected hypocotyls of *P. vulgaris* and elicitor-treated cell cultures with 32 P-labeled PAL and CHS cDNA sequences. Total cellular RNA (lanes 1, 3, and 5) and polysomal RNA (lanes 2 and 4) were isolated from site 1 hypocotyl tissue 69 h after inoculation with incompatible race β (lanes 1 and 2) and 150 h after inoculation with compatible race γ (lanes 3 and 4), as well as 3 h after elicitor treatment of suspension-cultured cells (lane 5). A 5- μ g sample of RNA was applied to each lane.

phytoalexin accumulation and the first visible sign of the flecking associated with expression of hypersensitive resistance). Accumulation of hybridizable PAL and CHS mRNAs was also observed in total cellular RNA isolated from sites 2 and 3, in uninfected tissue distant from the site of spore inoculation. mRNA accumulation in sites 2 and 3 occurred slightly later than accumulation in site 1 and was less pronounced, with a maximum 10- to 15-fold increase in the PAL mRNA level and a 20- to 30-fold increase in the CHS mRNA level compared with the levels in total cellular RNA samples from equivalent uninoculated hypocotyls.

In the compatible interaction with race γ , there was no significant increase in hybridizable PAL and CHS mRNA levels above the control levels during the early stages of infection equivalent to the phase in incompatible interactions of mRNA accumulation and expression of hypersensitive resistance (Fig. 3 through 5). Subsequently, however, at the start of lesion development, there was a marked increase in the hybridizable mRNA level correlated with the onset of phytoalexin accumulation during attempted lesion limitation. Accumulation of both PAL and CHS mRNAs was more pronounced and occurred slightly earlier in directly infected tissue at the site of spore inoculation (site 1), but there was also significant accumulation in site 3 and especially in site 2, in tissue distant from the initial site of infection (Fig. 3 and 4). The maximum levels of PAL and CHS mRNAs in the compatible interaction were severalfold greater than the levels in the incompatible interaction (Fig. 2). This reflected the fact that in the incompatible interaction only a small proportion of the cells became infected (1–3, 32) and presumably maximal activation of defense genes was restricted to these cells and their near neighbors (4).

The utilization of PAL and CHS mRNAs in protein synthesis was monitored by comparing the kinetics of accumulation of hybridizable PAL and CHS mRNAs in total cellular RNA with the kinetics of accumulation of hybridiz-

FIG. 3. Induction of PAL mRNA in hypocotyls of *P. vulgaris* cv. Kievitsboon Koekoek during race-cultivar-specific interactions with *C. lindemuthianum*. (A and B) Incompatible interaction (host resistant) with race β . (C and D) Compatible interaction (host susceptible) with race γ . Total cellular RNA (A and C) and polysomal RNA (B and D) were isolated from directly infected tissue (site 1) (●), from tissue laterally adjacent to the infected tissue (site 2) (▲), from tissue beneath sites 1 and 2 (site 3) (□), and from equivalent control uninoculated hypocotyls (site 4) (○). The dashed lines in panels B and D indicate the pattern of PAL synthesis in the site 1 tissue, as previously measured by *in vitro* translation of polysomal RNA and immunoprecipitation of [35 S]methionine-labeled enzyme subunits (8). The arrows in panel A indicate the following events in the expression of hypersensitive resistance at site 1: arrow a, spore

inoculation; arrow b, onset of hypersensitive flecking in a few sites; arrow c, onset of phytoalexin accumulation; arrow d, hypersensitive flecking apparent at most sites; arrow e, very dense brown flecking at all sites. No visible changes occurred in sites 2 through 4 throughout the time course. The arrows in panel C indicate the following events in lesion development at site 1: arrow a', spore inoculation; arrow b', no visible symptoms (cf. incompatible interaction); arrow c', onset of symptom development at a few sites; arrow d', onset of phytoalexin accumulation; arrow e', pale to mid-brown lesions apparent at most sites; arrow f', onset of water soaking and development of spreading lesions; arrow g', extensive water soaking and spreading of lesions from site 1 and some browning at site 2.

able PAL and CHS mRNAs in the polysomal RNA fraction and previously observed changes in the translatable activity of PAL and CHS mRNAs in isolated polysomal RNA (4, 8). In the compatible interaction there was a very close correlation between accumulation of hybridizable PAL and CHS mRNAs in the polysomal RNA fraction and accumulation in the total cellular RNA pool (Fig. 3 and 4). However, in the incompatible interaction, while changes in the levels of hybridizable PAL and CHS mRNAs in the polysomal RNA fraction followed broadly the same pattern as changes in the levels of hybridizable PAL and CHS mRNAs as a proportion of total cellular RNA, increased levels in site 1 were not observed until somewhat later in the polysomal RNA fraction than in total cellular RNA. Furthermore, for CHS mRNA but not PAL mRNA, elevated levels in sites 2 and 3 were more transient in the polysomal RNA fraction than in total cellular RNA (Fig. 4). While in the compatible interaction PAL and CHS mRNAs present in the polysomal RNA fraction appeared to account for most, if not all, of the hybridizable PAL and CHS mRNAs in total cellular RNA, in the incompatible interaction only a relatively small proportion of the total hybridizable PAL and CHS mRNAs was present in the polysomal RNA fraction (Fig. 2).

In directly infected tissue (site 1), during both compatible and incompatible interactions, changes in the rates of PAL and CHS polypeptide synthesis, as previously measured by *in vitro* translation of isolated polysomal RNA and immunoprecipitation of [35 S]methionine-labeled PAL and CHS subunits (4, 8), followed closely the kinetics for accumulation of hybridizable mRNA in the polysomal RNA fraction (Fig. 3 and 4). Previous *in vitro* translation-immunoprecipitation studies also demonstrated increased PAL and CHS synthesis in sites 2 and 3 in the later stages of the compatible interaction, but failed to reveal stimulation of enzyme synthesis in sites 2 and 3 of the incompatible interaction compared with uninoculated control tissue. This discrepancy with our observations could be explained by the greater sensitivity and lower background level associated with RNA blot hybridization compared with immunoprecipitation procedures, in which low signals may be masked by nonspecific coprecipitation of small amounts of unrelated contaminant polypeptides.

Stimulation of PAL and CHS polypeptide synthesis in tissue distant from the site of spore inoculation was confirmed by direct two-dimensional gel electrophoresis of the total set of [35 S]methionine-labeled *in vitro* translation products encoded by polysomal RNA isolated from site 1 and site 2 tissues. Two-dimensional gel electrophoresis of immunoprecipitated PAL and CHS subunits following *in vitro* translation of polysomal RNA isolated from elicitor-treated cell cultures revealed the synthesis of a characteristic set of

three major and two or three minor PAL isopolypeptides which were about the same size (M_r , 77,000) had different pI values (5) and a set of 8 to 10 CHS isopolypeptides (M_r , 42,000 to 43,000) (Fig. 6). Stimulation of the synthesis of these characteristic sets of PAL and CHS isopolypeptides in

elicitor-treated cells was demonstrated by direct two-dimensional gel electrophoresis of the total set of in vitro translation products encoded by polysomal RNA from elicitor-treated and control cells. Likewise, using this approach, we demonstrated stimulation of PAL and CHS synthesis in site 1 and site 2 infected hypocotyl tissue during both compatible and incompatible interactions (Fig. 6).

Marked increases in PAL and CHS enzyme activity and accumulation of phytoalexins can also be stimulated by excision wounding of bean hypocotyl tissue (42), as part of a general wound response (24). In view of the marked increases in PAL and CHS mRNA levels observed in response to infection, we also monitored changes in the levels of hybridizable PAL and CHS mRNAs following excision of small sections of hypocotyl tissue and incubation under sterile conditions in the absence of the elicitor. Excision caused marked, rapid, concomitant accumulation of PAL and CHS mRNAs (Fig. 7). Increased mRNA levels were observed within 2 h, and maximum levels were attained 8 to 12 h after excision; this was followed by decay to relatively low levels. Maximum accumulation of hybridizable mRNA was correlated with the phase of rapid increase in enzyme activity (data not shown).

DISCUSSION

The marked accumulation of PAL and CHS mRNAs from very low basal levels, taken together with the previous observation of stimulation of PAL and CHS gene transcription in elicitor-treated cell cultures, strongly suggests that there is transcriptional activation of these defense genes in wounded and infected hypocotyls. Moreover, there are clear temporal and spatial differences between the compatible interaction and the incompatible interaction in the pattern of PAL and CHS mRNA accumulation in response to infection. Particularly striking was the marked early accumulation of hybridizable mRNA in the incompatible interaction but not in the compatible interaction, even though in the latter case the fungus had invaded the host cells at that stage (1, 2, 32). This implies that accumulation of PAL and CHS mRNAs in directly infected tissue in the early stages of the incompatible interaction is a consequence of molecular recognition specified by the plant disease resistance genes and pathogen avirulence genes which underlie race-cultivar specificity (18, 38), rather than a response to nonspecific reactions arising from fungal ingress.

In both types of interaction there is a close correlation between accumulation of hybridizable mRNA and increased enzyme synthesis, leading to increased enzyme activity at the onset of phytoalexin accumulation (4, 8). Thus, induction of the genes encoding PAL and CHS is a key early component in the causally related sequence of events which leads to activation of the phytoalexin defense response both in the early stages of an incompatible interaction associated with

FIG. 4. Induction of CHS mRNA in hypocotyls of *P. vulgaris* cv. Kievitsboon Koekoek during race-cultivar-specific interactions with *C. lindemuthianum*. (A and B) Incompatible interaction (host resistant) with race β . (C and D) Compatible interaction (host susceptible) with race γ . Total cellular RNA (A and C) and

polysomal RNA (B and D) were isolated from directly infected tissue (site 1) (●), from tissue laterally adjacent to the infected tissue (site 2) (▲), from tissue beneath sites 1 and 2 (site 3) (■), and from equivalent control uninoculated hypocotyls (site 4) (○). The dashed lines in panels B and D indicate the pattern of CHS synthesis in the site 1 tissue as previously measured by in vitro translation of polysomal RNA and immunoprecipitation of [35 S]methionine-labeled enzyme subunits (4). The arrows in panels A and C indicate events at site 1 in the expression of hypersensitive resistance and in lesion development, respectively, as described in the legend to Fig. 3.

FIG. 5. Accumulation of hybridizable PAL mRNA (○) and CHS mRNA (●) in the early stages of infection of hypocotyls of *P. vulgaris* L. cv. Kievitsboon Koekoek with physiological races of *C. lindemuthianum*. (A) Incompatible interaction with race β . (B) Compatible interaction with race γ . (C) Uninfected hypocotyls.

expression of hypersensitive resistance and during the later stages of a compatible interaction associated with attempted lesion limitation. The rapid accumulation of PAL and CHS mRNAs in response to excision implies that following perception of appropriate signals, hypocotyl tissue is competent to respond by very rapid activation of defense genes. This suggests that the timing of the induction of PAL and CHS mRNAs in response to infection is determined by the time taken for fungal ingress and deployment of gene activation signals as a result of molecular recognition in the incompatible interaction and general trauma in the compatible interaction (2), rather than involvement of an extensive set of secondary events between signal perception and mRNA induction. In particular, following spore inoculation there is a period of 30 to 40 h during which the spores germinate and produce an infection peg before fungal hyphae come in contact with the first host epidermal cell (1–3, 32) and molecular recognition occurs in the incompatible interac-

tion. Therefore, induction of PAL and CHS mRNAs is extremely rapid in relation to the timing of this initial cell-cell contact and occurs well before the first signs of hypersensitive flecking and expression of hypersensitive resistance. These observations do not appear to be consistent with the hypothesis that induction of PAL and CHS mRNAs is causally preceded by hypersensitive cell death and expression of hypersensitive resistance (18), but instead suggest that molecular recognition leads to rapid activation of defense genes and hence expression of resistance.

In both types of interaction, intercellular transmission of the elicitation signal can be inferred from the accumulation of PAL and CHS mRNAs in apparently healthy, hitherto uninfected tissue distant from the initial site of fungal spore inoculation. Thus, in the compatible interaction, mRNA accumulation in sites 2 and 3 occurs at a stage when detailed cytological studies (32) have shown that fungus is not present in these sites but is found only in site 1. At this stage

FIG. 6. Two-dimensional gel electrophoresis of PAL polypeptides (upper panels) and CHS polypeptides (lower panels) synthesized *in vitro* by translation of isolated polysomal RNA. RNAs were isolated from cell cultures 3 h after elicitor treatment (A and B), unelicited control cell cultures (C), an incompatible interaction at site 1 (69 h) (D), an incompatible interaction at site 2 (69 h) (E), a compatible interaction at site 1 (150 h) (F), a compatible interaction at site 2 (150 h) (G), and equivalent uninfected control hypocotyl tissue (H). In panel A, PAL and CHS polypeptides were immunoprecipitated by monospecific antisera prior to electrophoretic analysis. In panels B through H the total set of *in vitro* translation products were subjected to electrophoresis, and the regions containing the PAL and CHS polypeptides are shown. The large arrowheads indicate PAL and CHS polypeptides, and the small arrowheads indicate sets of reference polypeptides.

in site 1 tissue there is extensive cell death in hitherto uninfected cells at the periphery of the developing lesion. Hence, accumulation of PAL and CHS mRNAs in sites 2 and 3 during the later stages of the compatible interaction may reflect the mediation of endogenous elicitors released following death of host cells and dissolution of cell walls associated with the breakdown of the biotrophic phase of fungal growth (1, 15, 21–23). Accumulation of PAL and CHS mRNAs in the later stages of the compatible interaction in hitherto uninfected cells ahead of the invading pathogen suggests a plausible mechanism for the process of attempted lesion limitation (1), which under appropriate physiological conditions can restrict the size of the lesion and prevent complete rotting of the organ and hence plant death, even though the interaction is genetically compatible.

Of particular interest is the observation of PAL and CHS mRNA accumulation in the early stages of the incompatible interaction not only in site 1, but also in sites 2 and 3. Expression of hypersensitive resistance is a localized event in terms of both restriction of fungal growth to a single host cell and the occurrence of host cell death and browning only in directly infected cells (12). However, prechallenge with an incompatible race of a pathogen leads to the establishment of induced systemic resistance to subsequent challenge by normally virulent compatible pathogens (11). The observa-

tion of induction of PAL and CHS mRNAs in hitherto uninfected cells distant from the initial site of infection by an incompatible pathogen may provide a clue to the molecular basis of such induced systemic resistance. Previous studies have shown that prechallenge of hypocotyls of *P. vulgaris* with an avirulent race does not lead to phytoalexin accumulation in distant tissue, but that following subsequent challenge inoculation with a normally virulent pathogen, there is a very rapid accumulation of phytoalexin which is reminiscent of an incompatible interaction (25). Likewise, expression of induced systemic resistance appears to involve more rapid lignification in response to challenge inoculation than is observed with equivalent nonimmunized tissue (25). Thus, the immunized state may reflect the accumulation of mRNAs encoding defense proteins which remain untranslated or are only weakly translated until a second, challenge inoculation, when these preexisting mRNAs are rapidly and efficiently incorporated into polysomes, leading to more rapid and effective activation of phytoalexin accumulation and lignin deposition than following infection of equivalent nonimmunized tissue with a compatible pathogen.

Evidence for the operation of posttranscriptional controls during the infection of bean hypocotyls with an incompatible race of *C. lindemuthianum* comes from our observation of differences in the kinetics and extent of accumulation of

FIG. 7. Accumulation of hybridizable PAL and CHS mRNAs in excised segments of hypocotyls of *P. vulgaris* L. The two zero-time samples represent RNAs isolated from intact unwounded hypocotyl tissue equivalent to the tissue from which excised sections were obtained at the start of the time course (0a) and equivalent unwounded hypocotyl tissue obtained 30 h later (0b).

hybridizable PAL and CHS mRNAs in the polysomal RNA fraction compared with the total cellular RNA. Thus, in the incompatible interaction, unlike the compatible interaction, considerable proportions of the total hybridizable PAL and CHS mRNAs are not present in the polysomal RNA fraction. Furthermore, both PAL mRNA accumulation and CHS mRNA accumulation occurs somewhat later in site 1, and CHS mRNA accumulation is more transient in sites 2 and 3 in the polysomal RNA fraction than as a proportion of total cellular RNA. Hence, although defense mRNAs accumulate in both directly infected tissue and distant uninfected tissue, efficient incorporation or prolonged incorporation of these mRNAs into polysomes might require secondary signals localized to directly infected cells and near neighbors in an incompatible interaction and challenged cells in immunized tissue. In contrast, the concomitant mRNA accumulation in the polysomal RNA fraction and total cellular RNA observed in the later stages of the compatible interaction might reflect simultaneous and widespread deployment of all signals needed for full activation of defense mechanisms in response to extensive traumatization of host tissue at the onset of lesion formation.

Parallel studies have shown that infection of hypocotyls causes a marked accumulation of transcripts encoding the apoproteins of hydroxyproline-rich glycoproteins in a temporal and spatial pattern broadly similar to that observed for PAL and CHS mRNAs (40). The picture beginning to emerge is that accumulation of defense gene transcripts characteristically underlies activation of plant defense responses and expression of disease resistance, thereby focusing attention on the organization and structure of defense genes in relation to activation by biological stress.

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