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Rapid transient induction of phenylalanine ammonia-lyase mRNA in elicitor-treated bean cells

(cDNA cloning/cDNA sequence/phytoalexins/plant disease resistance/thiouridine)

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ABSTRACT **DNAs complementary to a size-selected fraction of poly(A)$^+$ RNA present in elicitor-treated cells of bean (*Phaseolus vulgaris* L.) were inserted into pAT153 and used to transform *Escherichia coli* strain C600. Five clones were identified by hybrid-selected translation and cross-hybridization that contained sequences complementary to mRNA encoding phenylalanine ammonia-lyase (EC 4.3.1.5), which catalyzes the first reaction of phenylpropanoid biosynthesis. The longest insert contained a single open reading frame of 1520 base pairs together with 223 base pairs of 3′ untranslated sequence. RNA blot hybridization showed that elicitor caused a rapid, marked but transient increase in phenylalanine ammonia-lyase mRNA that was closely correlated with changes in translatable mRNA activity *in vitro* and enzyme synthesis *in vivo*. Blot hybridization of newly synthesized mRNA purified by organomercurial affinity chromatography following *in vivo* pulse-labeling with 4-thiouridine indicates that elicitor caused a rapid stimulation of phenylalanine ammonia-lyase mRNA synthesis as an early event in the defense response leading to accumulation of phenylpropanoid-derived phytoalexins.**

Phenylalanine ammonia-lyase (EC 4.3.1.5) is a key enzyme of plant metabolism catalyzing the first reaction in the biosynthesis from L-phenylalanine of a wide variety of natural products based on the phenylpropane skeleton (1). Fluctuations in phenylalanine ammonia-lyase levels are a major element in the control of phenylpropanoid biosynthesis in higher plants (1, 2). For example, enzyme levels are markedly increased by light concomitant with the onset of accumulation of flavonoids (3) or hydroxycinnamic acid esters (4), excision leading to wound-induced accumulation of hydroxycinnamic acid esters (4), and hormonal changes leading to lignification during xylem differentiation (5). Furthermore, phenylalanine ammonia-lyase activity is characteristically stimulated by microbial infection leading to the synthesis of lignin-like, wall-bound phenolic material and phenylpropanoid-derived phytoalexin antibiotics (1–3). These defense responses can also be induced by elicitors present in fungal cell walls and culture filtrates (2, 6–9).

Labeling *in vivo* and translation studies *in vitro* have shown environmental control over *de novo* synthesis of phenylalanine ammonia-lyase (1–3). In particular, marked increases in phenylalanine ammonia-lyase synthesis and translatable mRNA activity have been observed in response to elicitor treatment or infection in a number of systems (10–21). Thus, treatment of suspension-cultured bean (*Phaseolus vulgaris* L.) cells with an elicitor heat-released from cell walls of the fungus *Colletotrichum lindemuthianum* causes a rapid increase in the rate of synthesis of phenylalanine ammonia-lyase and at least three other enzymes of phenylpropanoid biosynthesis associated with the accumulation of wall-bound phenolic material and isoflavonoid phytoalexins such as phaseollin (refs. 10–13; unpublished data). The increase in phenylalanine ammonia-lyase synthesis from low basal rates reflects an increase in mRNA activity (13, 14), which, together with modulation of the apparent stability of the enzyme *in vivo* (11), is responsible for a marked stimulation of enzyme activity leading to increased phenylpropanoid biosynthesis and expression of the defense responses. Detailed physiological and cytological studies have established the importance of phytoalexin accumulation for disease resistance in bean (22).

Further investigation of the early molecular events underlying expression of disease resistance in biologically stressed cells and elucidation of the organization of phenylalanine ammonia-lyase genes in relation to activation by environmental factors requires specific hybridization probes. A cDNA clone containing sequences complementary to a phenylalanine ammonia-lyase mRNA that accumulates following illumination of parsley cells has been identified (23). In the present investigation we constructed a cDNA library complementary to size-selected poly(A)$^+$ RNA present in elicitor-treated bean cells. From this library a number of clones containing phenylalanine ammonia-lyase sequences have been identified, sequenced, and used to measure by RNA blot hybridization the kinetics of phenylalanine ammonia-lyase mRNA accumulation in response to elicitor.

MATERIALS AND METHODS

Enzyme Induction. Treatment of bean cell suspension cultures (24) with elicitor heat-released from mycelial cell walls (25) of *C. lindemuthianum* (26), extraction, and assay of phenylalanine ammonia-lyase activity (12) were as described. One unit of enzyme activity (1 kat) is defined as the amount of enzyme required for the formation of 1 mol of product in 1 sec under the assay conditions. Measurement of the rate of enzyme synthesis *in vivo* by incorporation of [^{35}S]methionine into immunoprecipitable phenylalanine ammonia-lyase subunits (12, 27) was as described.

Isolation of RNA. Polysomal RNA was isolated from cells by a modification (28) of the method of Palmiter (29). Total cellular RNA was isolated from cells homogenized directly in a phenol/0.1 M Tris·HCl, pH 9.0, emulsion as described by Haffner *et al.* (30). Further purification of the phenol-extracted total RNA was identical to that used for polysomal RNA. Newly synthesized RNA was isolated by two cycles of organomercurial affinity chromatography (Affi-Gel 501, Bio-Rad) from the total cellular RNA fraction derived from cells

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Abbreviation: bp, base pair(s).

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pulse-labeled with 4-thiouridine (1 mM) for 1 hr immediately prior to harvest as described (31).

Protein Synthesis *in Vitro*. Isolated polysomal RNA or total cellular RNA was translated *in vitro* in the presence of [^{35}S]methionine with an mRNA-dependent rabbit reticulocyte lysate translation system (32) and incorporation of [^{35}S]methionine into total protein was measured as described (14). Phenylalanine ammonia-lyase was separated from other translation products by indirect immunoprecipitation with monospecific antiserum to *P. vulgaris* phenylalanine ammonia-lyase and protein A-Sepharose followed by NaDodSO$_4$/polyacrylamide gel electrophoresis (27). Phenylalanine ammonia-lyase mRNA activity is defined as the incorporation of [^{35}S]methionine into immunoprecipitable enzyme subunits as a percentage of incorporation into total protein (14).

Cloning Procedure. Poly(A)$^+$ RNA (100 μg) from cells harvested 3 hr after elicitor treatment was fractionated by centrifugation at 40,000 $\times$ g_{av} for 16 hr at 4°C in a 5–30% sucrose gradient containing 0.1 M LiCl, 20 mM Tris·HCl (pH 7.2), and 10 mM vanadyl ribonucleoside complexes (Bethesda Research Laboratories). Fractions containing translatable phenylalanine ammonia-lyase mRNA activity were used to construct a cDNA library essentially as described (33) except that double-stranded cDNA was size-fractionated on a 1.5-ml Bio-Gel A-150m column. DNA larger than 600 base pairs (bp) was tailed with dC residues and inserted into dG-tailed pAT153 (34). The resulting plasmids were used to transform *Escherichia coli* strain C600 (35). Recombinants were picked into microtiter plates and were transferred subsequently to Biodyne nylon membranes (Pall, Portsmouth, United Kingdom) for colony hybridization.

Screening and Hybrid Selection of Phenylalanine Ammonia-Lyase mRNA. Plasmid DNA was prepared from those colonies showing hybridization to cDNA complementary to RNA from cells 3 hr after elicitor treatment but not to cDNA complementary to RNA from control unelicited cells. Plasmid (20 μg) was linearized by cleavage with *Hin*dIII and bound to 1-cm^2 Biodyne nylon membranes. Hybrid selection was performed as described (36) except that hybridization was at 50°C. Selected RNA was translated *in vitro* and the translation products were analyzed by immunoprecipitation with anti-phenylalanine ammonia-lyase serum and NaDodSO$_4$ gel electrophoresis as described above. DNA was sequenced by the dideoxy method (37) using M13 phages (38).

RNA Blot Hybridization. RNA (15 μg) was denatured with glyoxal, separated by electrophoresis on a 1.2% agarose gel in 10 mM phosphate buffer (pH 7.0) (39), and blotted onto nitrocellulose (40). The blots were hybridized with a probe prepared by nick-translation of the 800-bp insert fragment generated by digestion of pPAL1 with *Pst* I and separated from the vector by electroelution following gel electrophoresis. Hybridization was performed at 42°C in a buffer containing 50% formamide, 0.75 M NaCl, 0.075 M sodium citrate (pH 7.0), 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 5 mM EDTA, 0.1% NaDodSO$_4$, and 100 μg of calf thymus DNA per ml. Filters were preincubated for 24 hr in buffer without probe followed by hybridization for 48 hr in the presence of ^{32}P-labeled probe. Following autoradiography mRNA was quantitated by scanning densitometry calibrated by reference to internal standard phenylalanine ammonia-lyase mRNA samples.

RESULTS

A library of about 2500 colonies was constructed containing cDNA complementary to a size-selected fraction of poly(A)$^+$ RNA from elicitor-treated cells. By differential colony hybridization with cDNA probes complementary to RNA from elicitor-treated and control cells, about 80 colonies from this library were identified as containing elicitor-induced sequences. These clones were further characterized by restriction fragment analysis and hybrid selection. Clone CG12 was found to contain an 800-bp insert that hybrid selected mRNA

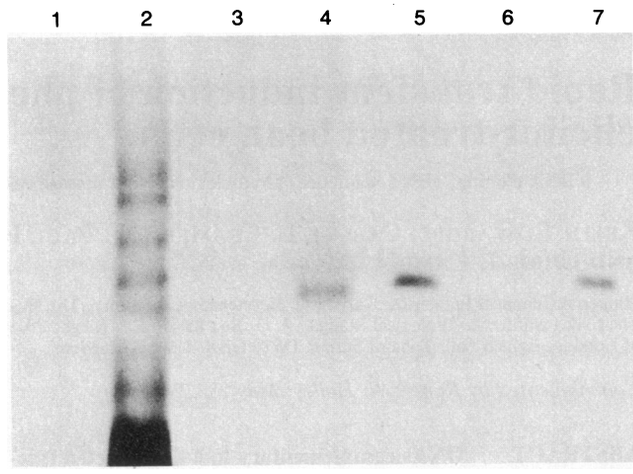


FIG. 1. Hybrid-selected translation with the insert of plasmid pPAL1. Substrates for the *in vitro* translations were derived from poly(A)$^+$ RNA isolated from bean cells 3 hr after elicitor treatment. Lane 1, no exogenous RNA control; lanes 2 and 5, total unselected poly(A)$^+$ RNA; lanes 3 and 6, RNA hybrid selected by pAT153 (vector); lanes 4 and 7, RNA hybrid selected by the plasmid carried by clone CG12 (pPAL1). For lanes 1–4, 5.0 μl of the respective ^{35}S-labeled polypeptide products was mixed with sample buffer and loaded directly onto the gel. For lanes 5–7, 15 μl of the respective ^{35}S-labeled polypeptide products was treated with monospecific antiserum to bean phenylalanine ammonia-lyase, and the immunoprecipitated material was resuspended in sample buffer and applied to the gel. The polypeptide immunoprecipitated by antiserum to phenylalanine ammonia-lyase (lane 7) accounted quantitatively for all of the polypeptide material encoded by mRNA hybrid selected specifically by the insert of pPAL1 (lane 4). The presence of antibody protein in samples run in lanes 5 and 7 slightly affected the mobility of phenylalanine ammonia-lyase subunits compared to lane 4.

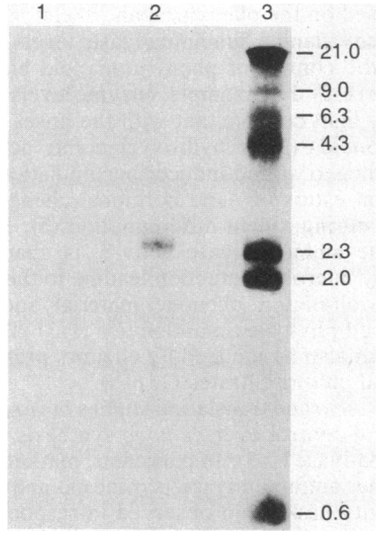
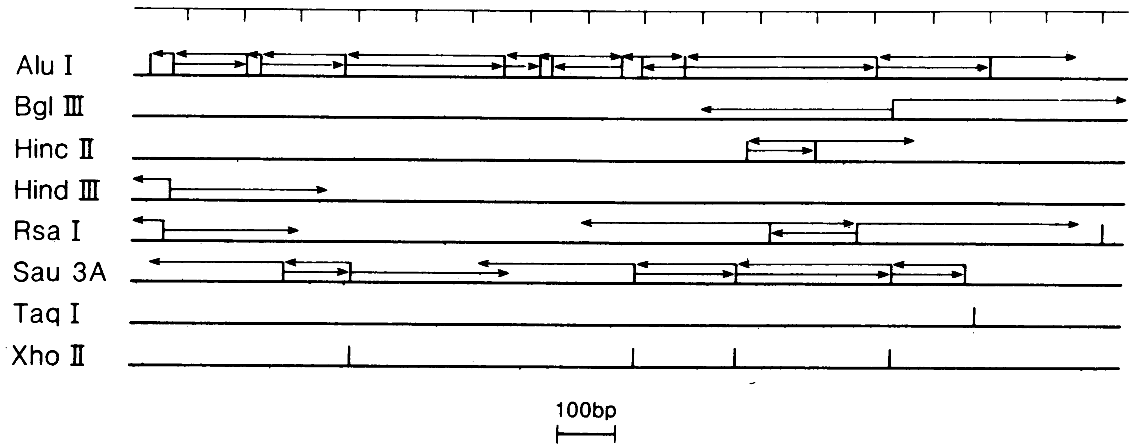


FIG. 2. Induction of phenylalanine ammonia-lyase mRNA in elicitor-treated bean cells. Total cellular RNA was isolated from untreated control cells (lane 1) and cells 3 hr after elicitor treatment (lane 2) and was fractionated by electrophoresis in agarose gels. After transfer to nitrocellulose, phenylalanine ammonia-lyase mRNA was detected by autoradiography following hybridization with [^{32}P]cDNA from the insert of pPAL1. mRNA size (given in kilobases) was determined by comparison with *Hin*dIII fragments of λ DNA (lane 3).

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     IleAlaGlyLeuLeuThrGlyArgProAsnSerLysAlaValGlyProSerGlyGluValLeuThrAlaLysGlnAlaPheGluLeu
1    GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGATTGCTGGTTTGCTCACTGGCAGACCTAACTCCAAAGCTGTTGGACCTTCTGGAGAAGTACTTACTGCTAAGCAAGCTTTTGAATTG

     AlaAsnIleAsnSerGluPheTyrGluLeuGlnProLysGluGlyLeuAlaLeuValAsnGlyThrAlaValGlySerGlyMetAlaSerIleValLeuPheAspAlaAsnIleLeuAla
121  GCTAACATCAATTCTGAGTTCTATGAATTGCAACCCAAGGAAGGCCTTGCCCTTGTTAATGGCACGGCTGTCGGTTCTGGCATGGCTTCTATAGTTCTCTTTGATGCAAATATACTAGCT

     ValLeuSerGluValLeuSerAlaIlePheAlaGluValMetGlnGlyLysProGluPheThrAspHisLeuThrHisLysLeuLysHisHisProGlyGlnIleGluAlaAlaAlaIle
241  GTGTTGTCTGAAGTTCTATCAGCTATTTTTGCCGAAGTGATGCAAGGGAAACCTGAGTTTACTGATCATTTGACACACAAGTTGAAGCACCACCCTGGTCAAATTGAGGCTGCTGCCATT

     MetGluHisIleLeuAspGlySerSerTyrMetLysAspAlaLysLysLeuHisGluIleAspProLeuGlnLysProLysGlnAspArgTyrAlaLeuArgThrSerProGlnTrpLeu
361  ATGGAGCACATTTTGGATGGAAGTTCCTACATGAAGGATGCTAAGAAGCTGCATGAAATAGATCCGTTGCAAAAGCCAAAACAAGATAGATATGCCCTCAGAACTTCACCACAGTGGCTT

     GlyProLeuIleGluValIleArgPheSerThrLysSerIleGluArgGluIleAsnSerValAsnAspAsnProLeuIleAspValSerArgAsnLysAlaLeuHisGlyGlyAsnPhe
481  GGTCCTCTCATTGAAGTCATTCGTTTCTCCACCAAGTCAATTGAGAGAGAGATTAACTCCGTGAATGACAACCCTCTTATTGATGTTTCAAGGAACAAGGCATTGCATGGTGGTAATTTC

     GlnGlyThrProIleGlyValSerMetAspAsnThrArgLeuAlaLeuAlaSerIleGlyLysLeuMetPheAlaGlnPheSerGluLeuValAsnAspPheTyrAsnAsnGlyLeuPro
601  CAAGGAACCCCAATTGGAGTCTCCATGGACAACACTCGTTTGGCTCTTGCATCTATCGGCAAACTCATGTTTGCTCAATTCTCCGAGCTTGTCAATGATTTCTACAACAACGGTCTGCCT

     SerAsnLeuThrAlaSerArgAsnProSerLeuAspTyrGlyPheLysGlyAlaGluIleAlaMetAlaSerTyrCysSerGluLeuGlnTyrLeuAlaAsnProValThrSerHisVal
721  TCAAATCTCACTGCTAGCAGAAATCCTAGCTTGGATTATGGTTTCAAGGGAGCTGAAATTGCCATGGCTTCCTATTGCTCTGAACTCCAGTATCTGGCAAATCCAGTAACAAGCCATGTC

     GlnSerAlaGluGlnHisAsnGlnAspValAsnSerLeuAspLeuIleSerAlaArgLysThrAsnGluSerIleGluIleLeuLysLeuMetSerSerThrPheLeuMetGlyLeuCys
841  CAAAGTGCTGAGCAACACAACCAAGATGTGAACTCTTTGGATTTGATTTCAGCTAGGAAGACAAATGAATCTATTGAGATCCTTAAGCTCATGTCTTCCACGTTCTTGATGGGACTTTGC

     GlnAlaIleAspLeuArgHisLeuGluGluAsnLeuLysSerSerValLysAsnThrValSerGlnValSerLysArgThrLeuThrThrGlyGlyAsnGlyGluLeuHisProSerArg
961  CAAGCAATTGACTTGAGGCATTTGGAGGAGAATTTGAAAAGCTCGGTCAAGAACACTGTGAGTCAAGTTTCCAAGAGGACTCTTACCACAGGTGGCAACGGAGAACTCCATCCTTCAAGA

     PheCysGluLysAspLeuLeuLysValValAspArgGluTyrValPheSerTyrIleAspAspProTyrSerGlyThrTyrProLeuMetGlnLysLeuArgGlnValLeuValAspHis
1081 TTTTGCGAAAAGGATCTGCTGAAAGTGGTTGACAGGGAGTATGTATTTTCCTACATAGATGACCCATACAGTGGTACATACCCACTGATGCAAAAACTTAGGCAAGTGCTTGTAGACCAT

     AlaLeuIleAsnAlaGluAsnGluLysAspValAsnThrSerIlePheGlnLysIleAlaThrPheGluGluGluLeuLysThrIleLeuProLysGluValGluSerThrArgAlaAla
1201 GCCTTGATAAATGCAGAGAATGAGAAGGATGTCAACACATCAATCTTTCAAAAGATTGCAACCTTTGAGGAGGAGTTGAAGACCATCTTGCCAAAGGAGGTTGAAAGTACAAGGGCTGCT

     TyrGluSerGlyLysAlaAlaIleProAsnLysIleLysGluCysArgSerTyrProLeuTyrLysPheValArgGluGluLeuGlyThrGlyLeuLeuThrGlyGluLysValLysSer
1321 TATGAGAGTGGGAAAGCTGCAATTCCAAACAAGATAAAAGAATGCAGATCTTACCCACTCTACAAGTTTGTGAGAGAGGAGTTGGGCACTGGGTTGCTAACTGGAGAAAAGGTGAAGTCA

     ProGlyGluGluPheAspLysLeuPheThrAlaIleCysGlnGlyLysIleIleAspProLeuLeuGluCysLeuGlyGluTrpAsnGlyAlaProLeuProIleCys
1441 CCAGGTGAAGAGTTTGACAAGTTATTCACAGCAATATGCCAGGGCAAAATTATTGATCCTCTTCTCGAATGCCTTGGAGAGTGGAATGGAGCTCCTCTTCCAATTTGTTGATTTTACTGT

1561 CATTTTTATAAGTATTTTTCTCTGTATCTACGCAAGTGGAAACCATAATCATCGTGCAGTTTGGCGTAAGCCTTTTAACAAATCTACATGGAAAGCCGGATTCTAATGTTTCCTCCATGT

1681 CAGATAGGACCTTGTAATTTAATATTATAGTGAAATTTCAGTTGATTAATGATTCGTACTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA   1775
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FIG. 3. Restriction map, sequencing strategy, and nucleotide sequence of the cDNA insert of pPAL5. The partial amino acid sequence of bean phenylalanine ammonia-lyase was deduced from the nucleotide sequences of the single open reading frame.

encoding a polypeptide that was immunoprecipitable by monospecific antiserum to bean phenylalanine ammonia-lyase and comigrated in $NaDodSO_4$/polyacrylamide gel electrophoresis with immunoprecipitated phenylalanine ammonia-lyase subunits (M_r 77,000) synthesized *in vitro* from poly(A)$^+$ RNA from elicitor-treated cells (Fig. 1). In RNA blot hybridization, nick-translated ^{32}P-labeled sequences of the plasmid insert of clone CG12 hybridized specifically to a single RNA species of about 2.5 kilobases that was highly induced by elicitor treatment (Fig. 2).

By these criteria clone CG12 was shown to carry a plasmid with an insert containing sequences complementary to bean phenylalanine ammonia-lyase mRNA and the plasmid was designated pPAL1. Rehybridization of the original library with an insert from pPAL1 revealed four additional clones containing phenylalanine ammonia-lyase sequences with inserts between 1500 bp and 1750 bp. Restriction fragment analysis failed to reveal sequence polymorphism between the five inserts. pPAL5 carries the largest insert, which contains only one open reading frame representing 1520 bp of the

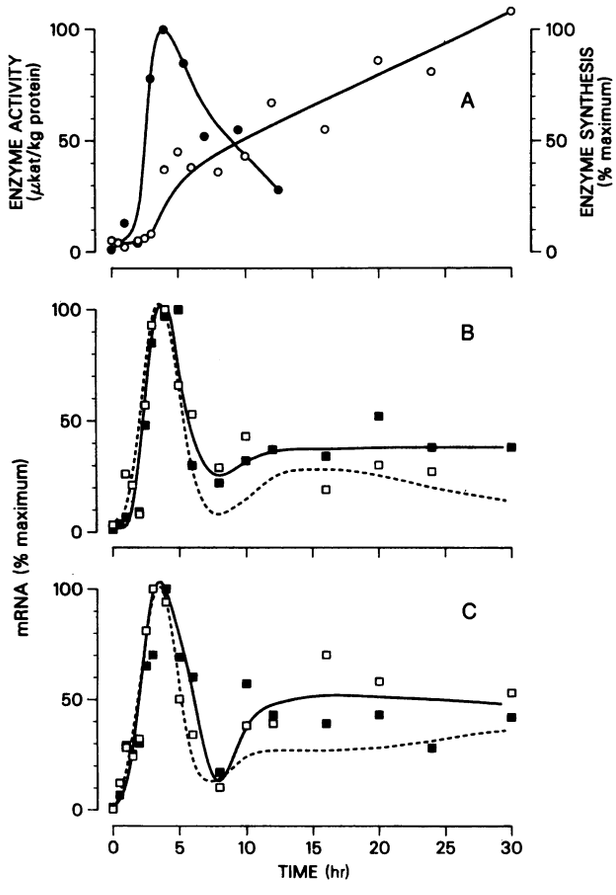

FIG. 4. Induction kinetics of phenylalanine ammonia-lyase in elicitor-treated bean cells. (*A*) Enzyme activity (○) and rate of enzyme synthesis *in vivo* (●). (*B*) Hybridizable mRNA (■) and translatable mRNA activity (□) in the polysomal RNA fraction. (*C*) Hybridizable mRNA (■) and translatable mRNA activity (□) in the total cellular RNA fraction. Enzyme synthesis, mRNA activity, and hybridizable mRNA are expressed relative to the respective maximal level attained during the time course. The dashed lines in *B* and *C* denote corresponding changes in hybridizable chalcone synthase mRNA as determined previously (41).

coding sequence for phenylalanine ammonia-lyase protein together with a 3′ untranslated region of 223 bp (Fig. 3).

The phenylalanine ammonia-lyase cDNA insert generated by digestion of pPAL1 with *Pst* I was separated from the cloning vehicle and used to measure the kinetics of phenylalanine ammonia-lyase mRNA accumulation in elicitor-treated cells (Fig. 4). Phenylalanine ammonia-lyase mRNA was almost completely absent from control cells but rapidly accumulated following elicitor treatment. Increases in phenylalanine ammonia-lyase mRNA could be observed 30 min after elicitation and maximal accumulation occurred 3–4 hr after elicitor treatment. Subsequently, the mRNA decayed rapidly to relatively low levels. Phenylalanine ammonia-lyase mRNA did not accumulate in mock-treated, unelicited control cells (data not shown).

Accumulation and decay of phenylalanine ammonia-lyase mRNA in total and polysomal RNA fractions exhibited very similar kinetics (Fig. 4). Furthermore, there was a close correlation between changes in hybridizable mRNA, translatable mRNA activity, and phenylalanine ammonia-lyase synthesis *in vivo*. Maximal stimulation coincided with the period of most rapid increase in phenylalanine ammonia-lyase enzyme activity.

The effect of elicitor on the synthesis of phenylalanine ammonia-lyase mRNA was monitored by blot hybridization

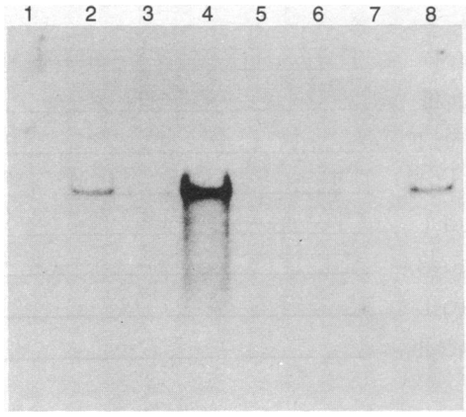

FIG. 5. Elicitor induction of the synthesis of phenylalanine ammonia-lyase mRNA. Unelicited control cells and cells 2.5 hr after elicitor treatment were labeled for 1 hr with 4-thiouridine. For measurement of mRNA levels, 12 μg of total cellular RNA samples (lanes 1, 2, 7, and 8) and 6 μg of affinity-separated RNAs (lanes 3, 4, 5, and 6) were fractionated by agarose gel electrophoresis prior to blot hybridization with ^{32}P-labeled bean phenylalanine ammonia-lyase sequences. Lanes 1 and 2, total cellular RNA from 4-thiouridine-labeled cells; lanes 3 and 4, 4-thiouridine-labeled, newly synthesized RNA selected by organomercurial affinity chromatography; lanes 5 and 6, unlabeled, preexisting RNA unbound during organomercurial affinity chromatography; lanes 7 and 8, total cellular RNA from equivalent unlabeled cells. Lanes 1, 3, 5, and 7 contain RNA isolated from unelicited control cells; lanes 2, 4, 6, and 8 contain RNA isolated from cells 3.5 hr after elicitor treatment.

of newly synthesized RNA purified by organomercurial affinity chromatography following *in vivo* pulse-labeling with 4-thiouridine. The relative abundance of phenylalanine ammonia-lyase mRNA in the fraction of newly synthesized RNA from cells pulse-labeled during the phase of rapid increase in mRNA 2.5–3.5 hr after elicitor treatment was ≈100-fold greater than in the equivalent fraction from unelicited control cells (Fig. 5).

DISCUSSION

The close correspondence between the kinetics for accumulation of translatable mRNA activity and hybridizable mRNA in both total cellular RNA and polysomal RNA fractions indicates that the transient increase in phenylalanine ammonia-lyase mRNA is the major factor governing the rate of enzyme synthesis throughout the phase of rapid increase in enzyme activity. The rapid increase from very low basal levels suggests that elicitor stimulates mRNA synthesis. This is confirmed by the abundance of 4-thiouridine-labeled, newly synthesized phenylalanine ammonia-lyase mRNA from elicitor-treated cells compared to equivalent, unelicited control cells, which can be taken as a measure of the relative rates of mRNA synthesis in elicited and control cells during the pulse-labeling period (31). Elicitor activation of phenylalanine ammonia-lyase gene transcription has been demonstrated recently by comparison of run-off transcripts in nuclei isolated from elicited and control cells (unpublished). Posttranscriptional control mechanisms may become important in the later stages of the response, especially in relation to the subsequent rapid decay in hybridizable phenylalanine ammonia-lyase mRNA.

The rapidity of mRNA induction and the close correlation with stimulation of enzyme synthesis implies that elicitor activation of phenylalanine ammonia-lyase genes is an early event in the causally related sequence of events in bean cells between elicitor binding to a putative receptor protein and expression of the defense response. The kinetics for elicitor induction of phenylalanine ammonia-lyase mRNA follow

very closely those observed for the mRNA encoding the phytoalexin biosynthetic enzyme chalcone synthase, which catalyzes the first reaction in a branch pathway of phenylpropanoid metabolism specific for flavonoid and isoflavonoid biosynthesis (41). This highly coordinated induction of mRNAs encoding two metabolically related enzymes strongly suggests that a similar mechanism operates for many, if not all, of the enzymes of phenylpropanoid phytoalexin biosynthesis. Furthermore, elicitor treatment also causes a marked accumulation of mRNAs encoding hydroxyproline-rich glycoproteins (42).

Recent studies have shown that elicitor treatment of parsley cell suspension cultures induces mRNAs encoding phenylalanine ammonia-lyase and 4-coumarate: CoA ligase, the third enzyme of the phenylpropanoid pathway (23, 43). However, in parsley, marked accumulation of hybridizable phenylalanine ammonia-lyase mRNA is not observed until 5 hr after elicitor treatment (23). This is after the period of rapid enzyme synthesis (3–4 hr after elicitor treatment) that regulates induction of enzyme activity and flux through the pathway leading to accumulation of furanocoumarin phytoalexins (44). Hence, mRNA induction by elicitor is apparently less rapid than might be expected if phenylalanine ammonia-lyase gene activation regulated expression of the phytoalexin defense response in this system. Despite this anomaly, the overall picture that begins to emerge from these studies is that rapid, selective changes in the pattern of gene expression characteristically underlie activation of plant defense responses.

Recent work has demonstrated at least four forms of native phenylalanine ammonia-lyase in suspension-cultured bean cells (27). These multiple forms, which exhibit different isoelectric points and kinetic properties, are differentially induced by elicitor. Similarly, two-dimensional gel electrophoresis of immunoprecipitated enzyme subunits, synthesized *in vivo* in elicitor-treated cells or synthesized *in vitro* from total cellular RNA isolated from elicited cells, has revealed the presence of multiple subunit forms of about the same size (M_r 77,000) but with different isoelectric points (27). Identification of cDNA clones containing sequences complementary to bean phenylalanine ammonia-lyase mRNA now provides the basis for analysis of the organization and structure of phenylalanine ammonia-lyase genes in relation to this polymorphism and specific activation by elicitor, infection, and other environmental factors.

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1. Jones, D. H. (1984) *Phytochemistry* **23**, 1349–1360.
2. Dixon, R. A., Dey, P. M. & Lamb, C. J. (1983) *Adv. Enzymol. Relat. Areas Mol. Biol.* **55**, 1–136.
3. Hahlbrock, K. & Grisebach, H. (1979) *Annu. Rev. Plant Physiol.* **30**, 105–130.
4. Lamb, C. J. & Rubery, P. H. (1976) *Phytochemistry* **15**, 665–668.
5. Haddon, L. E. & Northcote, D. H. (1976) *Planta* **128**, 255–262.
6. Pearce, R. B. & Ride, J. P. (1982) *Physiol. Plant Pathol.* **20**, 119–123.
7. West, C. A. (1981) *Naturwissenschaften* **68**, 447–457.
8. Darvill, A. G. & Albersheim, P. (1984) *Annu. Rev. Plant Physiol.* **35**, 243–275.
9. Robbins, M. P., Bolwell, G. P. & Dixon, R. A. (1985) *Eur. J. Biochem.* **148**, 571–578.
10. Dixon, R. A. & Lamb, C. J. (1979) *Biochim. Biophys. Acta* **586**, 453–463.
11. Lawton, M. A., Dixon, R. A. & Lamb, C. J. (1980) *Biochim. Biophys. Acta* **633**, 162–175.
12. Lawton, M. A., Dixon, R. A., Hahlbrock, K. & Lamb, C. J. (1983) *Eur. J. Biochem.* **129**, 593–601.
13. Cramer, C. L., Bell, J. N., Ryder, T. B., Bailey, J. A., Schuch, W., Bolwell, G. P., Robbins, M. P., Dixon, R. A. & Lamb, C. J. (1985) *EMBO J.* **4**, 285–289.
14. Lawton, M. A., Dixon, R. A., Hahlbrock, K. & Lamb, C. J. (1983) *Eur. J. Biochem.* **130**, 131–139.
15. Hahlbrock, K., Lamb, C. J., Purwin, C., Ebel, J., Fautz, E. & Schäfer, E. (1981) *Plant Physiol.* **68**, 680–685.
16. Loschke, D. C., Hadwiger, L. A. & Wagoner, W. (1983) *Physiol. Plant Pathol.* **23**, 163–173.
17. Börner, H. & Grisebach, H. (1982) *Arch. Biochem. Biophys.* **217**, 65–71.
18. Ebel, J., Schmidt, W. E. & Loyal, R. (1984) *Arch. Biochem. Biophys.* **232**, 240–248.
19. Schmelzer, E., Börner, H., Grisebach, H., Ebel, J. & Hahlbrock, K. (1984) *FEBS Lett.* **172**, 59–63.
20. Duchesne, M., Fritig, B. & Hirth, L. (1977) *Biochim. Biophys. Acta* **485**, 464–481.
21. Loschke, D. C., Hadwiger, L. A., Schröder, J. & Hahlbrock, K. (1981) *Plant Physiol.* **68**, 680–685.
22. Bailey, J. A. (1982) in *Active Defense Mechanisms in Plants*, ed. Wood, R. K. S. (Plenum, New York), pp. 39–65.
23. Kuhn, D. H., Chappell, J., Boudet, A. & Hahlbrock, K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1102–1106.
24. Dixon, R. A. & Bendall, D. S. (1978) *Physiol. Plant Pathol.* **13**, 295–306.
25. Bailey, J. A. & Deverall, B. J. (1971) *Physiol. Plant Pathol.* **1**, 435–449.
26. Anderson-Prouty, A. J. & Albersheim, P. (1975) *Plant Physiol.* **56**, 286–306.
27. Bolwell, G. P., Bell, J. N., Cramer, C. L., Schuch, W., Lamb, C. J. & Dixon, R. A. (1985) *Eur. J. Biochem.* **149**, 411–419.
28. Schröder, J., Betz, B. & Hahlbrock, K. (1976) *Eur. J. Biochem.* **67**, 527–541.
29. Palmiter, R. D. (1974) *Biochemistry* **13**, 3606–3615.
30. Haffner, M. H., Chin, M. B. & Lane, B. G. (1978) *Can. J. Biochem.* **56**, 729–733.
31. Cramer, C. L., Ryder, T. B., Bell, J. N. & Lamb, C. J. (1985) *Science* **227**, 1240–1243.
32. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256.
33. Bartels, D. & Thompson, R. D. (1983) *Nucleic Acids Res.* **11**, 2961–2977.
34. Twigg, A. J. & Sheratt, D. (1980) *Nature (London)* **283**, 216–218.
35. Geiser, M., Doring, H., Westemeyer, J., Behrens, U., Tillmann, E. & Starlinger, P. (1980) *Nucleic Acids Res.* **8**, 6175–6187.
36. Wood, D., Blanchlot, A. & Jeffreys, A. J. (1982) *Nucleic Acid Res.* **10**, 7133–7144.
37. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
38. Messing, J., Crea, R. & Seeburg, P. H. (1981) *Nucleic Acids Res.* **9**, 309–321.
39. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4835–4839.
40. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
41. Ryder, T. B., Cramer, C. L., Bell, J. N., Robbins, M. P., Dixon, R. A. & Lamb, C. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5724–5728.
42. Showalter, A. M., Bell, J. N., Cramer, C. L., Bailey, J. A., Varner, J. E. & Lamb, C. J. (1985) *Proc. Natl. Acad. Sci. USA*, in press.
43. Chappell, J. & Hahlbrock, K. (1984) *Nature (London)* **311**, 76–78.
44. Tietjen, K. G., Hunkler, D. & Matern, U. (1983) *Eur. J. Biochem.* **131**, 401–407.