Differential induction of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase genes in *Arabidopsis thaliana* by wounding and pathogenic attack

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ABSTRACT   We have isolated cDNAs from two distinct ᵥ genes encoding 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase (EC 4.1.2.15) in *Arabidopsis thaliana*. Predicted protein sequences from both genes, DHS1 and DHS2, and a potato DAHP synthase gene are highly related, but none shows significant sequence similarity to conserved microbial DAHP synthase proteins. Despite this structural difference, the DHS1 cDNA complements mutations in a yeast strain lacking DAHP synthase activity. DHS1 RNA levels increase in *Arabidopsis* leaves subjected either to physical wounding or to infiltration with pathogenic *Pseudomonas syringae* strains. DHS2 RNA levels are not increased by these treatments, suggesting that the DHS1 and DHS2 proteins fulfill different physiological functions. Other enzymes in the *Arabidopsis* aromatic pathway are also encoded by duplicated genes, an arrangement that may allow independent regulation of aromatic amino acid biosynthesis by distinct physiological requirements such as protein synthesis and secondary metabolism. The presence of amino-terminal extensions characteristic of chloroplast transit peptides on DHS1 and DHS2 suggests that both proteins may be targeted to the chloroplast.

The regulation of aromatic amino acid biosynthesis in plants must accommodate the requirements of both protein synthesis and secondary metabolism. Aromatic amino acids are precursors to a diverse array of plant secondary metabolites, including lignin, anthocyanic pigments, auxin, and antimicrobial phytoalexins (1, 2). These compounds are involved in many processes, including growth control (auxin), physical support and water relations (lignin), and resistance to infection (phytoalexins), and their synthesis appears to be developmentally and environmentally regulated. The functional diversity of secondary metabolites suggests that the synthesis of each may be under separate regulatory control. The biosynthesis of aromatic amino acids in plants may, in turn, require complex regulatory controls that differ fundamentally from those described for bacteria and fungi.

This report describes the structure and expression of two *Arabidopsis thaliana* genes encoding 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase [7-phospho-2-dehydro-3-deoxy-D-*arabino*-heptonate D-erythrose-4-phosphate-lyase (pyruvate-phosphorylating); EC 4.1.2.15], the enzyme catalyzing the first committed step in aromatic amino acid biosynthesis. These genes may have distinct physiological roles, as they are differentially expressed in plants subjected either to physical wounding or to infiltration by virulent and avirulent strains of *Pseudomonas syringae*. Genes encoding other enzymes in the *Arabidopsis* aromatic pathway are also duplicated (refs. 3 and 4; K. Niyogi and G.R.F., unpublished work). We suggest that duplication of the genes encoding these enzymes, and possibly all enzymes in the pathway, provides a mechanism to permit independent regulation of aromatic amino acid biosynthesis by different physiological demands, such as protein synthesis and secondary metabolism. The presence of sequences encoding putative chloroplast transit peptides on all *Arabidopsis* aromatic-pathway genes analyzed to date suggests that differentially regulated aromatic amino acid biosynthesis pathways may exist within the chloroplast.¶

MATERIAL AND METHODS

Isolation of *Arabidopsis* DAHP Synthase cDNA Clones. Clones were isolated from a cDNA library (5) by hybridization to a labeled fragment of a potato DAHP synthase gene (6). The probe was prepared by PCR amplification of potato genomic DNA using oligonucleotide primers corresponding to coding-strand nucleotides 16–36 (5'-GCAATGGCTCTTTCAAG-TACT-3') and to noncoding-strand nucleotides 903–882 (5'-ACCAAGGGCCTCATCCACTCTA-3') of λBD21 (6). Filters containing phage plaques were hybridized at 58°C in 5× standard saline/phosphate/EDTA (SSPE) and washed at 50°C in 1× standard saline citrate (SSC). cDNA inserts from positive clones were subcloned into pBluescript KS(−) (Stratagene) and sequences of nested deletions were determined (7). Analyses of DAHP synthase sequences from *Escherichia coli* [aroF (8), aroG (9), and aroH (10)], *Saccharomyces cerevisiae* [ARO3 (11)], potato (6), and *Arabidopsis* were performed using the University of Wisconsin Genetics Computer Group programs BESTFIT, IALIGN (12), and MALI (13). The sequence similarity values shown in Table 1 were obtained using the program BESTFIT (12), and the alignment shown in Fig. 1 was made using the program MALI (13).

Blot Hybridization of *Arabidopsis* DNA and RNA. *Arabidopsis* genomic DNA was digested with restriction enzymes (New England Biolabs), electrophoresed in a 0.7% agarose gel, and blotted to nitrocellulose membranes. Hybridization probes were prepared by random oligonucleotide labeling (Prime Time kit, IBI) of full-length DHS1 and DHS2 cDNAs. Blots were hybridized in 5× SSPE/5× Denhardt's solution/0.1% SDS at 68°C and washed in 0.1× SSC/0.1% SDS at 65°C (14).

Total RNA was isolated from *Arabidopsis* plants by miniprep procedures (15, 16). RNA was electrophoresed in formaldehyde/1% agarose gels (14) and blotted to nitrocellulose or GeneScreen (New England Nuclear) membranes. Hybridization was performed in either 50% formamide/5× SSPE/0.1% SDS/5× Denhardt's solution at 42°C (14) or 0.5 M Na_2HPO_4, pH 7.2/7% SDS/1% bovine serum albumin at 60°C (17). Blots were washed in 0.2× SSC/0.1% SDS at 55°C. Autoradiographs were scanned with a computing densitometer (Mo-

lecular Dynamics, Sunnyvale, CA) to obtain approximate values for changes in transcript levels.

Complementation of Yeast DAHP Synthase Mutation. *S. cerevisiae* strain YBK7 (*MATa, aro3-2, aro4-1, ura3-1,* Gal⁺) was transformed with plasmids as described (18).

Wounding and Bacterial Infiltration of *Arabidopsis* Plants. Three-week-old plants were wounded by two methods: (*i*) rosette leaves were diced with scissors and kept in a darkened humidified chamber or (*ii*) leaves were scored with a metal file and left attached to the plant. Both methods produced the same patterns of DAHP synthase gene expression. Bacterial infiltration of *Arabidopsis* plants was achieved as described (19).

RESULTS

Structure of *Arabidopsis* DAHP Synthase Genes. *Arabidopsis* DAHP synthase cDNA clones *DHS1* and *DHS2* were identified by cross-hybridization to a potato DAHP synthase gene fragment (6). As shown in Fig. 1 and Table 1, protein sequences deduced from *DHS1, DHS2,* and potato DAHP synthase cDNAs are largely identical, except in their amino-terminal portions. These divergent regions are characteristic of chloroplast transit peptides; they are rich in serine, threonine, and proline residues and carry a net positive charge (20). Southern blot analysis of *Arabidopsis* genomic DNA hybridized to *DHS1* and *DHS2* cDNA probes revealed cross-hybridization between *Arabidopsis* DHS genes (Fig. 2A). Neither probe hybridized consistently to other genomic fragments under low-stringency conditions (data not shown). Restriction fragment length polymorphism mapping revealed that *DHS1* and *DHS2* genes are linked, separated by ≈27 centimorgans; these data extend the existing map of chromosome 4 (Fig. 2B).

The polypeptide sequences deduced from the *Arabidopsis* and potato DAHP synthase genes display a high degree of similarity, but none of these genes shows significant sequence homology to the highly conserved DAHP synthase protein sequences of *E. coli* or *S. cerevisiae* (Table 1). Attempts to identify *Arabidopsis* genes homologous to microbial DAHP synthase genes by low-stringency hybridization and PCR techniques (22) were not successful.

Complementation of Yeast DAHP Synthase Mutations. To demonstrate that the *Arabidopsis* genes encode functional DAHP synthase enzymes, we used the *DHS1* cDNA to complement mutations in yeast strain YBK7. YBK7 is incapable of growth on synthetic complete medium lacking phenylalanine, tryptophan, and tyrosine, because of mutations in the two yeast DAHP synthase genes, *ARO3* and *ARO4*. The

full-length *DHS1* cDNA insert was placed under the control of the inducible *GAL1* promoter in the yeast expression vector pDAD2 and transformed into YBK7. Complementation of the *aro3* and *aro4* mutations in YBK7 by the GAL-DHS1 construct is expected to result in growth on medium containing galactose but lacking aromatic amino acids (Gal, −Aro). Initially, however, transformants containing the GAL-DHS1 construct did not display complementation. We observed complementation by the *DHS1* cDNA in segregants of the original transformants after subsequent selections. Individual transformants carrying the various pDAD2-derived plasmids were grown under uracil selection in liquid galactose medium to induce the *GAL1* promoter, and 10⁸ cells were plated on galactose medium lacking uracil and aromatic amino acids. No growth was observed on plates containing cells carrying the pDAD2 vector alone or the GAL-DHS1(antisense) construct. However, 100–200 slowly growing colonies appeared when cells containing the GAL-DHS1 construct were plated. Growth was due to the expression of the *DHS1* cDNA, as the same segregants were unable to grow on −Aro medium containing glucose, which represses *GAL1* promoter activity (Fig. 3).

Subsequent tests indicated that these Aro⁺ cells were segregants expressing sufficient *Arabidopsis* DAHP synthase activity to complement the yeast *aro3* and *aro4* mutations. First, segregants that lost the GAL-DHS1 plasmid became Aro⁻ on galactose. Second, the Aro⁺ phenotype was not a consequence of chromosomal mutation in the yeast strain, because segregants that lost the plasmid (Ura⁻, Aro⁻) again produced Aro⁺ revertants at a frequency of roughly 10⁻⁶ when retransformed with the GAL-DHS1 construct. Third, the Aro⁺ phenotype did not result from plasmid rearrangements, because plasmids isolated from the Aro⁺ segregants had the same properties as the original plasmid. The simplest explanation of our observations is that the Aro⁺ segregants arose by increased plasmid copy number. In agreement with this explanation, the Aro⁺ segregants were extremely unstable under nonselective conditions, segregating a high proportion of Ura⁺, Aro⁻ cells. We assume that these Aro⁻ segregants, which still retained the plasmid, simply had lowered copy number.

***DHS1* and *DHS2* Are Expressed Differently in Response to Wounding.** Intact *Arabidopsis* leaves contained approximately equal steady-state levels of *DHS1* and *DHS2* RNAs (Fig. 4A). After wounding, however, *DHS1* RNA levels increased about 3- to 5-fold, reaching a maximum at ≈1.5 hr. The level of *DHS2* RNA appeared to decline during the same time period. The level of transcripts encoding phenylalanine am-
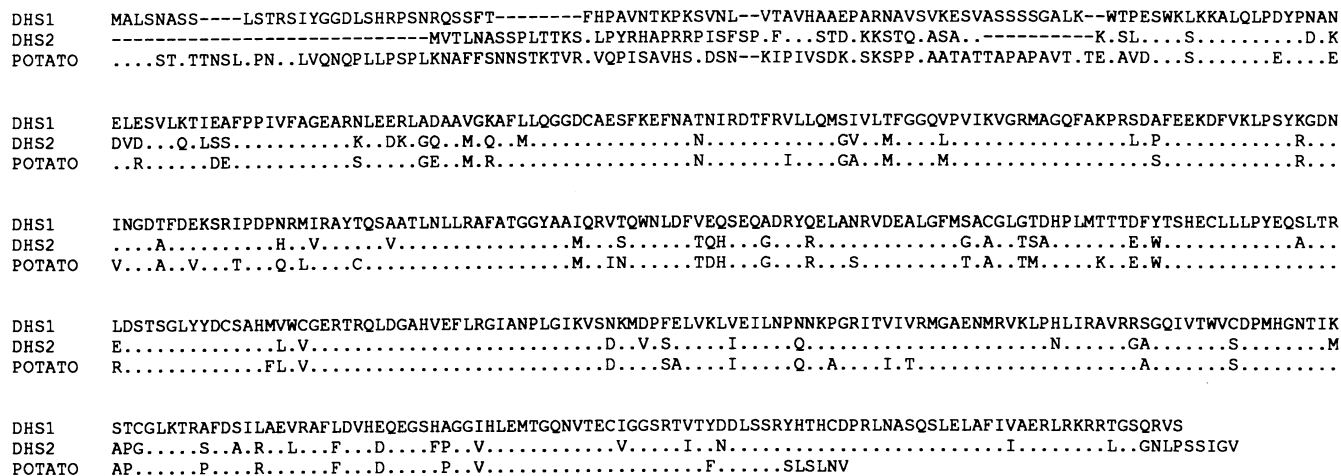
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DHS1    MALSNASS----LSTRSIYGGDLSHRPSNRQSSFT-------FHPAVNTKPKSVNL--VTAVHAAEPARNAVSVKESVASSSSGALK--WTPESWKLKKALQLPDYPNAN
DHS2    --------------------------MVTLNASSPLTTKS.LPYRHAPRRPISFSP.F...STD.KKSTQ.ASA..---------K.SL....S.........D.K
POTATO  ....ST.TTNSL.PN..LVQNQPLLPSPLKNAFFSNNSTKTVR.VQPISAVHS.DSN--KIPIVSDK.SKSPP.AATATTAPAPAVT.TE.AVD...S.......E....E


DHS1    ELESVLKTIEAFPPIVFAGEARNLEERLADAAVGKAFLLQGGDCAESFKEFNATNIRDTFRVLLQMSIVLTFGGQVPVIKVGRMAGQFAKPRSDAFEEKDFVKLPSYKGDN
DHS2    DVD...Q.LSS..........K..DK.GQ..M.Q..M..............N...........GV..M....L...............L.P...........R...
POTATO  ..R......DE..........S.....GE..M.R..................N.......I....GA..M....M..................S...........R...


DHS1    INGDTFDEKSRIPDPNRMIRAYTQSAATLNLLRAFATGGYAAIQRVTQWNLDFVEQSEQADRYQELANRVDEALGFMSACGLGTDHPLMTTTDFYTSHECLLLPYEQSLTR
DHS2    ....A..........H..V......V................M...S......TQH...G...R.............G.A..TSA.......E.W............A...
POTATO  V...A..V...T...Q.L....C..................M..IN......TDH...G...R...S.........T.A..TM.....K..E.W...............


DHS1    LDSTSGLYYDCSAHMVWCGERTRQLDGAHVEFLRGIANPLGIKVSNKMDPFELVKLVEILNPNNKPGRITVIVRMGAENMRVKLPHLIRAVRRSGQIVTWVCDPMHGNTIK
DHS2    E...............L.V.......................D..V.S.....I.....Q...............N......GA.......S........M
POTATO  R............FL.V.......................D....SA....I.....Q..A....I.T....................A.......S.........


DHS1    STCGLKTRAFDSILAEVRAFLDVHEQEGSHAGGIHLEMTGQNVTECIGGSRTVTYDDLSSRYHTHCDPRLNASQSLELAFIVAERLRKRRTGSQRVS
DHS2    APG.....S..A.R..L...F....D.....FP..V...........V.....I..N...........................I.........L..GNLPSSIGV
POTATO  AP......P....R......F...D.....P..V..................F......SLSLNV
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Fig. 1. Alignment of *Arabidopsis* (DHS1 and DHS2) and potato DAHP synthase amino acid sequences. Dots represent identity with DHS1 sequence. Potato DAHP synthase amino acid sequence was obtained from Dyer *et al.* (6).

Table 1. Plant and microbial DAHP synthase amino acid sequence similarity

	Arabidopsis DHS2	Potato λBD21	E. coli AroF	E. coli AroG	E. coli AroH	Yeast ARO3
DHS1	85 (76)	82 (73)	35 (13)	35 (13)	30 (11)	36 (16)
DHS2		86 (79)	35 (12)	39 (12)	35 (13)	35 (15)
λBD21			37 (14)	32 (11)	30 (13)	37 (16)
AroF				71 (53)	67 (46)	71 (49)
AroG					72 (56)	72 (55)
AroH						63 (43)

Numbers not in parentheses represent percent similarity; numbers in parentheses represent percent identity (see *Materials and Methods*).

monia lyase (PAL), the first enzyme in the phenylpropanoid pathway, also increased about 2- to 3-fold after wounding. The difference between our results and those of Ohl *et al.* (26), who observed induction of PAL RNA 1 hr after wounding *Arabidopsis* plants, may be due to differences in the manner and severity of the wounding procedure. Not all amino acid biosynthetic pathways respond to wounding, because transcripts from *ALS*, the single-copy *Arabidopsis* gene encoding acetolactate synthase (25), an enzyme in the isoleucine–valine pathway, showed the same pattern of expression as *DHS2* (Fig. 4A). The decline of *DHS2* and *ALS* RNA levels reflects general RNA degradation in wounded tissues. By 4 hr after wounding, the RNA samples were obviously degraded, as evidenced by increased mobility of hybridizing RNA in all samples. Induction of DAHP synthase RNA levels in wounded tissue has been observed in potato (27), although the number of potato DAHP synthase genes and their individual expression patterns have not been described.

Induction of *DHS1* RNA Levels by Pathogenic Attack. *DHS1* RNA levels were also induced about 3-fold in *Arabidopsis* plants challenged with bacterial pathogens. The virulent strain *Pseudomonas syringae* pv. *maculicola* ES4326 (Psm ES4326) multiplies 10^4- to 10^5-fold within 96 hr in infiltrated

Arabidopsis leaves, producing chlorotic lesions (19). In contrast, the avirulent strain *P. syringae* pv. *tomato* 1065 (Pst MM1065) does not multiply in infiltrated *Arabidopsis* leaves. Another avirulent strain, ES4326/pMMXR1, was created by transfer of a cloned avirulence (*avr*) gene from strain Pst MM1065 into strain Psm ES4326 (19). This strain induces a hypersensitive response in infiltrated *Arabidopsis* leaves (19). *DHS1* RNA levels increased ≈3-fold 6 hr after *Arabidopsis* plants were infiltrated with the avirulent strains Pst MM1065 or ES4326/pMMXR1 (Fig. 4B), whereas plants infiltrated with the virulent strain Psm ES4326 showed an increase 24 hr after infection. *DHS2* RNA levels did not show this induction following bacterial infiltration. Mock-infected plants showed no significant alteration of *DHS1* or *DHS2* RNA levels. Again, *ALS* transcripts were unaffected by these treatments (data not shown).

DISCUSSION

The predicted protein sequences from the *Arabidopsis* DAHP synthase cDNAs are markedly different from their microbial counterparts. Nevertheless, the *Arabidopsis DHS1* cDNA complements mutations in the yeast *ARO3* and *ARO4* genes. As complementation was observed only in segregants and not in the primary transformants, it is unlikely that these
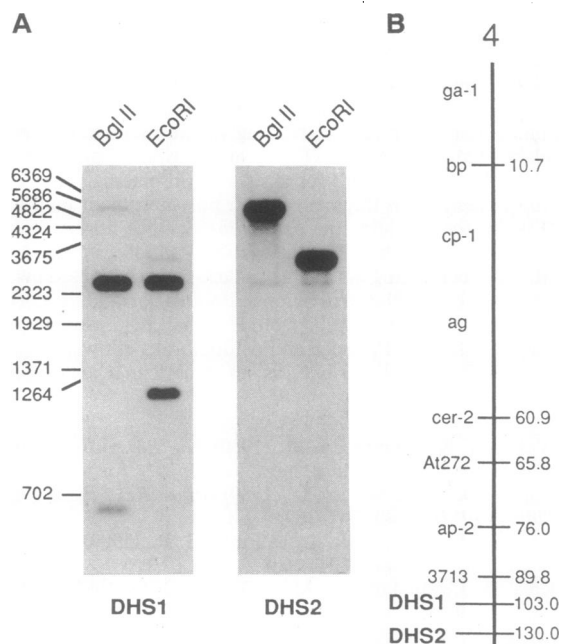

FIG. 2. (A) Southern blot analysis of *Arabidopsis* genomic DNA hybridized to *DHS1* and *DHS2* cDNA probes. Size markers are in base pairs. (B) Schematic representation of the *Arabidopsis* fourth chromosome, showing the positions of *DHS1* and *DHS2* in relation to other genetic markers. Symbols to the left of the vertical line represent mapped restriction fragment length polymorphisms and visible gene markers, numbers to the right represent map distances in centimorgans (21).
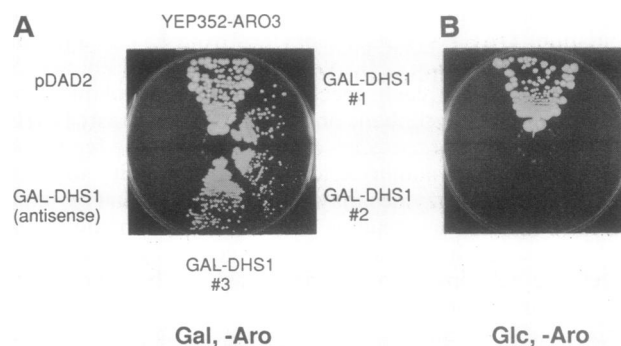

FIG. 3. Complementation of yeast strain YBK7 (*MAT*a, *aro3-2*, *aro4-1*, *ura3-1*, *GAL*⁺). (A) Growth of YBK7 transformed with plasmids on synthetic complete medium lacking phenylalanine, tryptophan, tyrosine, and uracil, supplemented with galactose (Gal, −Aro). The expression plasmid pDAD2 was created by introducing a polylinker and the *PHO5* terminator downstream of the *GAL1* promoter in pCGS109 (a gift of J. Schaum and J. Mao, Collaborative Research) (D. Miller, D. Pellman, and G.R.F., unpublished data). GAL-DHS1 and GAL-DHS1(antisense) constructs were created by cloning the 1.9-kilobase-pair *Eco*RI fragment containing the entire *DHS1* cDNA in the forward and reverse orientation, respectively, downstream of the *GAL1* promoter. Aro⁺ segregants of YBK7 carrying the GAL-DHS1 construct were isolated as described in the text and three independent clones were restreaked on the plates shown here. As a positive control, a 1.7-kilobase-pair *Hin*dIII–*Xba* I fragment containing the *ARO3* gene (23), under the control of its own promoter, was cloned into plasmid YEP352 (24) and transformed into YBK7. (B) Growth of the same transformed YBK7 strains on selective medium supplemented with glucose (Glc, −Aro) instead of galactose.
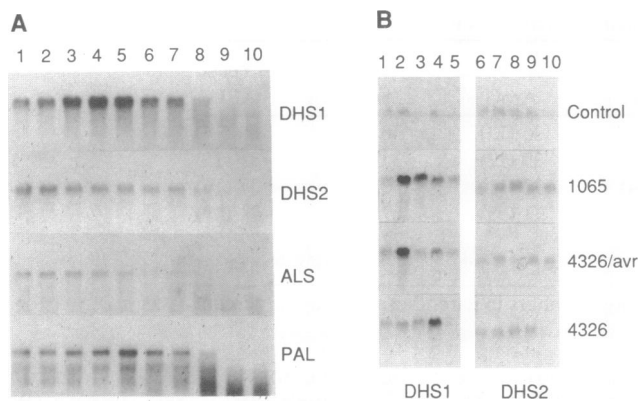
FIG. 4. Induction of *DHS1* RNA by wounding and bacterial infiltration. (*A*) RNA samples extracted from wounded *Arabidopsis* plants were hybridized to probes from *Arabidopsis DHS1, DHS2, ALS* (25), and *PAL* (19) genes. Five micrograms of total RNA was loaded in each lane. Lanes 1–10 contained samples obtained from plants 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 hr after wounding, respectively. The same blot was hybridized to all four probes. (*B*) Induction of *DHS1* RNA in *Arabidopsis* plants infiltrated with pathogenic *P. syringae* strains. *Arabidopsis* plants were infiltrated with avirulent strains Pst MM1065 or Psm ES4326/pMMXR1 (labeled 4326/avr), or with the virulent strain Psm ES4326 (19). Control plants were infiltrated with a 10 mM MgCl₂ solution. Five micrograms of total RNA was loaded in each lane. RNA samples were obtained 0 hr (lanes 1 and 6), 6 hr (lanes 2 and 7), 12 hr (lanes 3 and 8), 24 hr (lanes 4 and 9), and 48 hr (lanes 5 and 10) after infiltration. The same blot was hybridized to each probe.

cDNAs would have been identified by direct complementation from a transformed cDNA library. The poor complementing activity of the GAL-DHS1 construct may be due to inhibitory effects of the chloroplast transit-peptide sequence on expression of particular plant cDNAs in microbial backgrounds (28).

The different primary structures of plant and microbial DAHP synthase proteins may reflect differences in metabolic regulation. DAHP synthase isozymes from *E. coli* and *Salmonella typhimurium* (29), *Neurospora crassa* (30), and *S. cerevisiae* (31) are each specifically feedback-inhibited by phenylalanine, tryptophan, or tyrosine. In contrast, DAHP synthases from plant species do not appear to be feedback-inhibited by these amino acids (32, 33). Activity of plant DAHP synthase enzymes may be regulated instead by intermediates in pathways leading from the free aromatic amino acid to auxins, phenolics, and other secondary metabolites.

Defense responses to wounding and pathogenic attack involve the induction of PAL and other enzymes specific to lignin synthesis and secondary metabolism (34). Consequently, coordinate induction of aromatic amino acid biosynthesis may be required to supply aromatic precursors for the synthesis of defensive secondary metabolites. The relatively early induction of *DHS1* and *PAL* expression (19) by avirulent *Pseudomonas* strains may be critical for the timely production of compounds necessary to contain bacterial proliferation. In *Arabidopsis* and other Brassicaceae, these compounds may include sulfur-containing aromatic glucosinolates believed to function as part of a general defense system (35, 36). The belated increase in *DHS1* RNA levels in response to infiltration with the virulent Psm ES4326 strain may reflect a delayed recognition process or stress caused by disease progression. Induction of only one of the two *Arabidopsis* DAHP synthase genes by either wounding or bacterial infection indicates a clear difference in the regulation of the two genes and implies DHS1 as the major isozyme responding to changes in secondary metabolite biosynthesis.

Genes encoding four *Arabidopsis* aromatic amino acid biosynthetic enzymes have now been cloned: DAHP syn-

thase, tryptophan synthase B (3), 3-*enol*-pyruvoylshikimate-5-phosphate synthase (4), and anthranilate synthase (K. Niyogi and G.R.F., unpublished work); each is duplicated. Duplication of the entire *Arabidopsis* aromatic pathway at the genetic and enzymatic level may allow independent regulation of aromatic amino acid synthesis in response to distinct physiological requirements such as secondary metabolism and protein synthesis. Previous suggestions of duplicated aromatic pathways in plants (37) were based on biochemical separation of two pathway enzyme activities, DAHP synthase and chorismate mutase, into chloroplastic and cytosolic isoforms displaying different regulatory properties (33, 38, 39). While plastids appear to contain all the enzymes required for aromatic amino acid biosynthesis (37), the absence of cytosolic activities corresponding to other pathway enzymes argues against the existence of a complete aromatic pathway in the cytosol (40, 41). In parsley, chloroplast-associated DAHP synthase activity is induced when cells are treated with fungal elicitors, while the cytosol-associated activity remains unchanged (42). By analogy, our expression data predict that *DHS1* corresponds to the chloroplastic form and *DHS2* to the cytosolic. However, the putative chloroplast transit peptides on both DHS1 and DHS2 suggest that if *Arabidopsis* has a cytosolic DAHP synthase isozyme, it must either be encoded by a distinct gene or result from an alteration of the DHS1 or DHS2 protein. We have not detected additional *DHS* genes in low-stringency hybridization experiments.

Our data suggest that duplicate, independently regulated aromatic amino acid biosynthetic pathways may reside within the chloroplast. This idea is supported by the fact that all cloned *Arabidopsis* aromatic amino acid biosynthetic genes encode putative chloroplast transit peptides (refs. 3 and 4; K. Niyogi and G.R.F., unpublished work). Independent regulation may also involve different cell-type-specific expression patterns for each member of a gene pair, as observed for pea glutamine synthetase genes in transgenic tobacco (43). Transgenic plants containing *DHS* promoter sequences fused to reporter genes can address this issue.

1. Weiss, U. & Edwards, J. M. (1980) *The Biosynthesis of Aromatic Compounds* (Wiley, New York).
2. Hahlbrock, K. & Scheel, D. (1989) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**, 347–369.
3. Berlyn, M. B., Last, R. L. & Fink, G. R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4604–4608.
4. Klee, H. J., Muskopf, Y. M. & Gasser, C. S. (1987) *Mol. Gen. Genet.* **210**, 437–442.
5. Elledge, S. J., Mulligan, J. T., Ramer, S. W., Spottswood, M. & Davis, R. W. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1731–1735.
6. Dyer, W. E., Weaver, L. M., Zhao, J. M., Kuhn, D. N., Weller, S. C. & Herrmann, K. M. (1990) *J. Biol. Chem.* **265**, 1608–1614.
7. Sanger, F., Nicklen, S. & Coulsen, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
8. Shultz, J., Hermodson, M. A., Garner, C. C. & Herrmann, K. M. (1984) *J. Biol. Chem.* **259**, 9655–9661.

9. Davies, W. D. & Davidson, B. E. (1982) *Nucleic Acids Res.* 10, 4045–4058.
10. Zurawski, G., Gunsalus, R. P., Brown, K. D. & Yanofsky, C. (1981) *J. Mol. Biol.* 145, 47–73.
11. Paravicini, G., Braus, G. & Hutter, R. (1988) *Mol. Gen. Genet.* 214, 165–169.
12. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* 1, 387–395.
13. Vingron, M. & Argos, P. (1989) *Comput. Appl. Biosci.* 5, 115–121.
14. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
15. Nagy, F., Kay, S. A. & Chua, N.-H. (1988) in *Plant Molecular Biology Manual*, eds. Gelvin, S. B. & Schilperoort, R. A. (Kluwer, Boston), pp. B4/1–29.
16. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1990) *Current Protocols in Molecular Biology* (Greene/Wiley-Interscience, New York).
17. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* 81, 1991–1995.
18. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* 153, 163–168.
19. Dong, X., Mindrinos, M., Davis, K. R. & Ausubel, F. M. (1991) *Plant Cell* 3, 61–72.
20. von Heijne, G. (1990) *Curr. Opin. Cell. Biol.* 2, 604–608.
21. Nam, H.-G., Giraudat, J., den Boer, B., Moonan, F., Loos, W. D. B., Hauge, B. M. & Goodman, H. M. (1989) *Plant Cell* 1, 699–705.
22. Gould, S. J., Subramani, S. & Scheffler, I. E. (1989) *Proc. Natl. Acad. Sci. USA* 86, 1934–1938.
23. Teshiba, S., Furter, R., Niederberger, P., Braus, G., Paravicini, G. & Hutter, R. (1986) *Mol. Gen. Genet.* 205, 353–357.
24. Hill, J. E., Myers, A. M., Koerner, T. J. & Tzagoloff, A. (1986) *Yeast* 2, 163–167.

25. Mazur, B. J., Chui, C. F. & Smith, J. K. (1987) *Plant Physiol.* 85, 1110–1117.
26. Ohl, S., Hedrick, S. A., Chory, J. & Lamb, C. J. (1990) *Plant Cell* 2, 837–848.
27. Dyer, W. E., Henstrand, J. M., Handa, A. K. & Herrmann, K. M. (1989) *Proc. Natl. Acad. Sci. USA* 86, 7370–7373.
28. Wiersma, P. A., Schmiemann, M. G., Condie, J. A., Crosby, W. L. & Moloney, M. M. (1989) *Mol. Gen. Genet.* 219, 413–420.
29. Pittard, A. J. (1987) in *Escherichia coli and Salmonella typhimurium*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington), pp. 368–394.
30. Nimmo, G. A. & Coggins, J. R. (1981) *Biochem. J.* 199, 657–665.
31. Takahashi, M. & Chan, W. W. C. (1971) *Can. J. Biochem.* 49, 1015–1025.
32. Gilchrist, D. G. & Kosuge, T. (1980) in *The Biochemistry of Plants*, eds. Stumpf, P. K & Conn, E. E. (Academic, New York), pp. 507–531.
33. Rubin, J. L. & Jensen, R. A. (1985) *Plant Physiol.* 79, 711–718.
34. Bowles, D. J. (1989) *Annu. Rev. Biochem.* 59, 873–907.
35. Hogge, L. R., Reed, D. W., Underhill, E. W. & Haughn, G. W. (1988) *J. Chromatogr. Sci.* 26, 551–556.
36. Thangstad, O. P., Iversen, T.-H., Slupphaug, G. & Bones, A. (1990) *Planta* 180, 245–248.
37. Jensen, R. A. (1986) *Rev. Adv. Phytochem.* 20, 57–82.
38. Ganson, R. J., D'Amato, T. A. & Jensen, R. A. (1986) *Plant Physiol.* 82, 203–210.
39. d'Amato, T. A., Ganson, R. J., Gaines, C. G. & Jensen, R. A. (1984) *Planta* 162, 104–108.
40. Kishore, G. M. & Shah, D. M. (1988) *Annu. Rev. Biochem.* 57, 627–663.
41. Last, R. L., Bissinger, P. H., Mahoney, D., Radwanski, E. R. & Fink, G. R. (1991) *Plant Cell* 3, 345–358.
42. McCue, K. F. & Conn, E. E. (1989) *Proc. Natl. Acad. Sci. USA* 86, 7374–7377.
43. Edwards, J. W., Walker, E. L. & Coruzzi, G. M. (1990) *Proc. Natl. Acad. Sci. USA* 87, 3459–3463.