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Modes of expression and common structural features of the complete phenylalanine ammonia-lyase gene family in parsley

(cinnamate 4-hydroxylase/4-coumarate:CoA ligase/fungal elicitor/UV light/wounding)

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ABSTRACT **We describe a complete gene family encoding phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) in one particular plant species. In parsley (*Petroselinum crispum*), the PAL gene family comprises two closely related members, PAL1 and PAL2, whose TATA-proximal promoter and coding regions are almost identical, and two additional members, PAL3 and PAL4, with less similarity to one another and to the PAL1 and PAL2 genes. Using gene-specific probes derived from the 5′ untranslated regions of PAL1/2, PAL3, and PAL4, we determined the respective mRNA levels in parsley leaves and cell cultures treated with UV light or fungal elicitor and in wounded leaves and roots. For comparison, the functionally closely related cinnamate 4-hydroxylase (C4H) and 4-coumarate:CoA ligase (4CL) mRNAs were measured in parallel. The results indicate various degrees of differential responsiveness of PAL4 relative to the other PAL gene family members, in contrast to a high degree of coordination in the overall expression of the PAL, C4H, and 4CL genes. The only significant sequence similarities shared by all four PAL gene promoters are a TATA-proximal set of three putative cis-acting elements (boxes P, A, and L). None of these elements alone, or the promoter region containing all of them together, conferred elicitor or light responsiveness on a reporter gene in transient expression assays. The elements appear to be necessary but not sufficient for elicitor- or light-mediated PAL gene activation, similar to the situation previously reported for 4CL.**

Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) catalyzes the conversion of L-phenylalanine into *trans*-cinnamate, the first committed step of the multibranched phenylpropanoid metabolism in higher plants. PAL was described in 1961 by Koukol and Conn (1) and has since been one of the most extensively studied enzymes in plants. Various structural and regulatory properties of PAL genes from bean (2–4), rice (5), parsley (6, 7), *Arabidopsis* (8, 9), potato (10), loblolly pine (11), pea (12, 13), tomato (14), and poplar (15) have been reported. As far as analyzed, the number of PAL genes per haploid plant genome varies from 1 (11) to 5 (14), except for potato, which contains an unusually large family of ≈40–50 PAL genes (10). The most frequently occurring number of PAL genes is 3–4.

In none of these cases has the regulation of a complete gene family been investigated. This is the subject of the present study. Variously treated organs and suspension-cultured cells of parsley were chosen as a suitable system for investigating PAL gene expression, for several reasons. First, PAL has been studied extensively in parsley (16, 17) and has been shown to be encoded by a family of four distinct genes (6, 7). Second, while the enzyme-kinetic properties of all four encoded isoforms were almost indistinguishable (18), preliminary results indicated a certain degree of differential gene expression (17, 19). And finally, parsley is the only plant species for which homologous cDNA probes are also available for the two subsequent steps in phenylpropanoid metabolism, those catalyzed by cinnamate 4-hydroxylase (C4H; EC 1.14.13.11; previously unpublished results) and 4-coumarate:CoA ligase (4CL; EC 6.2.1.12; ref. 20). This latter aspect was of particular importance, because the three functionally closely related enzymes, PAL, C4H, and 4CL, have so far always been found to be coordinately regulated (16). It was therefore of interest to compare the expression modes of the individual PAL genes with C4H and 4CL gene expression. Furthermore, the two 4CL genes existing in parsley have been investigated extensively with respect to promoter structure and function (20–23) and will therefore serve in the following as a valuable reference for analysis of the four PAL gene promoters.

MATERIALS AND METHODS

Plants and Cell Cultures. Parsley plants (*Petroselinum crispum*, cv. Hamburger Schnitt) were grown for 6 months under greenhouse conditions (19). Cell suspension cultures were propagated in the dark and used 6 days after subculturing for treatments as described elsewhere (19, 20).

Molecular Biological Methods. Previously published methods were used for cloning of cDNA (24), using mixed mRNA preparations from cells treated for 0.5, 1.5, and 3 h with elicitor, and of genomic DNA (6), for DNA sequencing (24),‡ transcription start site determination (20), RNA extraction (6), Northern blot hybridization (6), synthesis of promoter fusion constructs (24), and protoplast transformation and β-glucuronidase (GUS) assays (25). The following promoter fragments were fused with the GUS reporter gene: a 168-bp fragment from the parsley PR2 promoter (26) and tetramerized versions of "unit 1" (containing boxes I and II) from the parsley chalcone synthase (CHS) promoter (24), of boxes P, A, and L from the parsley PAL1 promoter, of mutated box L ("Lm"; ref. 17), and of the PAL1 promoter regions containing either boxes P and A ("P/A") or boxes P, A, and L ("P/A/L"). The mung bean C4H cDNA (27) used for isolation of a near full-length parsley C4H cDNA was a kind gift of D. Ohta (Takarazuka, Japan).

RESULTS

Structural Analysis of the TATA-Proximal PAL Gene Promoters and 5′ Untranslated Regions. Near-full-length PAL2

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Abbreviations: C4H, cinnamate 4-hydroxylase; CHS, chalcone synthase; 4CL, 4-coumarate:CoA ligase (AMP-forming); GUS, β-glucuronidase; PAL, phenylalanine ammonia-lyase; PR2, pathogenesis-related protein 2; TS, transcription start.

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‡The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L37355, L37356, and L37357 for 5′ untranslated and TATA-proximal promoter regions of PAL2, PAL3, and PAL4, respectively, and L38898 for near-full-length C4H cDNA).

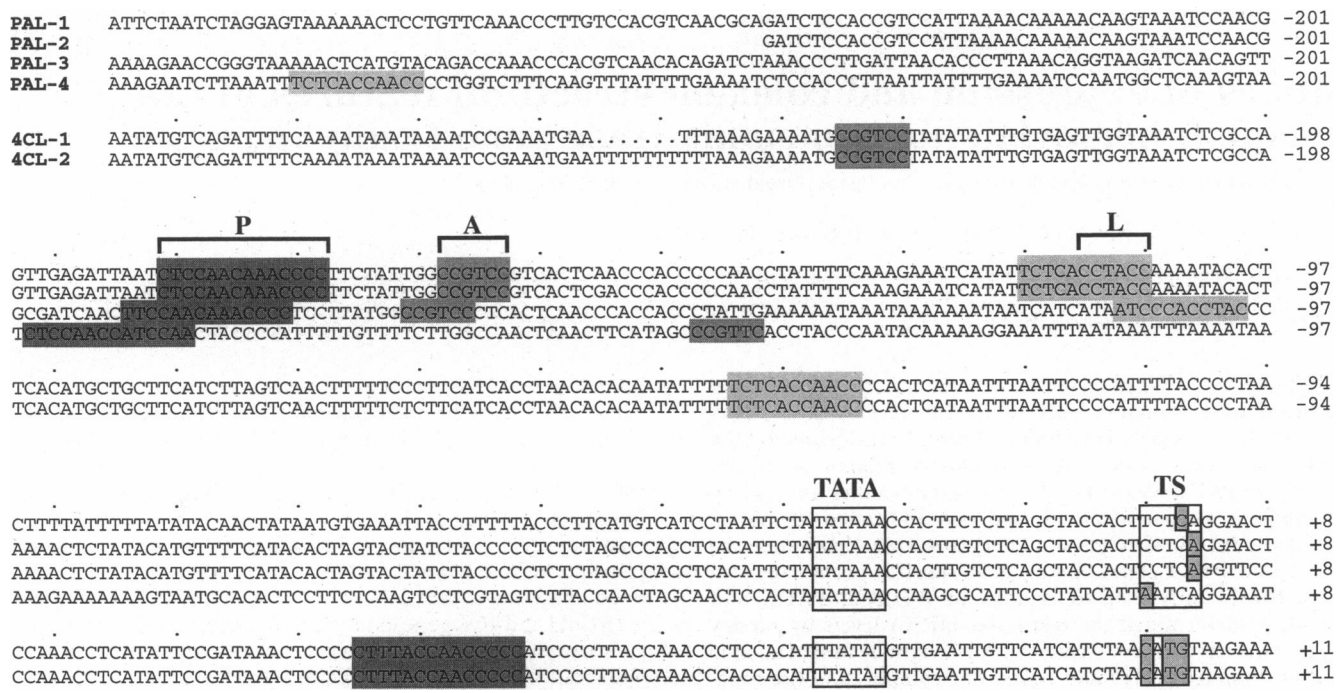

FIG. 1. TATA-proximal nucleotide sequences of the PAL and 4CL gene families in parsley. Brackets indicate previously established *in vivo* DNA footprints on the PAL1 gene promoter (6), subsequently designated as boxes P, A, and L (17, 28). Corresponding, differently shaded areas denote putative cis-acting elements common to all six gene promoters. Putative TATA boxes and relative locations of transcription start sites (TS, experimentally determined positions shaded) are shown in boxes. See text for explanation of numbering.

and PAL3 (18) as well as PAL4 (7) cDNAs were used to isolate and assign the respective cloned genes from a parsley genomic library. Their TATA-proximal promoter portions and 5′ untranslated regions were sequenced and compared with the corresponding, previously reported nucleotide sequence of the PAL1 gene (6). Transcription start (TS) sites of the PAL2, PAL3, and PAL4 genes were determined by S1 nuclease mapping and primer-extension analysis. They fell within a 5-bp region (TS box) located at the same distance from the respective TATA boxes in all parsley PAL and 4CL genes (Fig. 1). To facilitate direct comparison, the same numbering as reported previously for PAL1 (6) is used here for the nucleotide sequences of all four PAL genes (fourth position of TS box given as +1), whereas the numbering of the two 4CL genes remains as published earlier (20). When this numbering is used for alignment, the four PAL genes are found to be remarkably similar in nucleotide sequence around the TATA and TS boxes (positions −33 to +20) but differ greatly further upstream and downstream, except for PAL1 and PAL2, which are almost identical throughout the regions analyzed. Like the two 4CL gene promoters (20), the PAL1 and PAL2 gene promoters differ by no more than two nucleotides within the established

Table 1. Putative cis-acting elements and their positions on various PAL and 4CL gene promoters

Promoter*	Box P	Position†	Box A	Position†	Box L	Position†
*Pc*PAL1	CTCCAACAAACCCC	−175	CCGTCC	−160	TCTCACCTACC	−107
*Pc*PAL2	CTCCAACAAACCCC	−175	CCGTCC	−160	TCTCACCTACC	−107
*Pc*PAL3	TTCCAACAAACCCC	−178	CCGTCC	−163	TCCCACCTACC	−98
*Pc*PAL4	CTCCAACCATCCAA	−183	CCGTTC	−136	TCTCACCAACC	−268
*Pc*4CL1	CTTTACCAACCCCC	−52	CCGTCC	−228	TCTCACCAACC	−128
*Pc*4CL2	CTTTACCAACCCCC	−52	CCGTCC	−228	TCTCACCAACC	−128
*At*PAL1	TCTCAACCAACTCC	−135	CCGTGT	−260	GCTTACCTACC	−51
*At*PAL2	TCTCACCCACCCCT	−132	?‡		CCTTACCTAAC	−55
*Le*PAL5	TTCCTACAACCCCC	?§	?§		CTTTACCTACC	?§
*Ps*PAL1	ATTCAACAAACCAC	−136	CCATCC	−265	CCTCACCTACC	−92
*Ps*PAL2	CTCAACCAAACCAC	−204	CGCTCC	−227	CTTTACCTACC	−151
*Pv*PAL2	TCTCCACCAACCCC	−123	ACGTCC	−274	ACCCACCTACC	−76
*Pv*PAL3	CAACCACCTACCCC	−77	?‡		ACTCACCAACC	−53
*St*PAL1	TTCCAACAACCACC	−165	CCCTCT	−136	TCCCATCTCCA	−83
*St*PAL2	CTTCAACAACCACC	−169	CCCTCC	−140	TCTCATCTACC	−72
*St*4CL1	CTTTCACCTACCAC	−57	CTGTCC	−476	TCTCACCAACC	−170
*St*4CL2	CTTTCACCTACCAC	−58	CTGTCC	−476	TCTCACCAACC	−168
Consensus	C/T T C T C C A A A C C C C		CCGTCC		T C C T C A C C T A C C C C T A	

*At, *Arabidopsis thaliana* (8, 9); *Le, Lycopersicon esculentum* (14); *Pc, Petroselinum crispum* (this work and ref. 6); *Ps, Pisum sativum* (12, 13); *Pv, *Phaseolus vulgaris* (2); and *St, Solanum tuberosum* (10).

†Position relative to TS site.

‡Not within positions −1 to −473 (*At*) or −447 (*Pv*).

§Short promoter fragment, TS site not determined.

portion of the PAL2 gene promoter (up to position −242). A slightly larger difference between PAL1 and PAL2 (four nucleotides) was noted for the 5′ untranslated regions.

Within the regions analyzed (up to positions −1000 for PAL1, −242 for PAL2, −1000 for PAL3, and −978 for PAL4), the only apparent similarities (by computer analysis and visual inspection) shared by all four parsley PAL gene promoters upstream from the TATA boxes were three short nucleotide sequences previously identified by *in vivo* DNA footprinting as putative cis-acting elements (6). The same three elements, designated as boxes P, A, and L (17, 28), are also present, albeit at various relative locations, in the TATA-proximal promoter regions of both parsley 4CL genes (Fig. 1) and of nearly all other known PAL and 4CL genes. As shown in Table 1, deviations from the total set of the three derived consensus sequences average around two positions.

Timing of mRNA Induction. We used the 5′ untranslated regions of PAL1/2, PAL3, and PAL4 as gene-specific DNA probes (≈150–180 bp) to measure the respective mRNAs individually on Northern blots. Analogous to PAL1 and PAL2, which were detected together (PAL1/2) by the PAL1 probe, the sum of the 4CL1 and 4CL2 mRNAs (4CL1/2) was monitored by using the 4CL1 cDNA (20). Since no homologous C4H probe was available at the beginning of this study, we prepared a near-full-length parsley C4H cDNA, using a mung bean C4H probe for screening of a cDNA library (82.5% identity and 93% similarity of the deduced amino acid sequences).

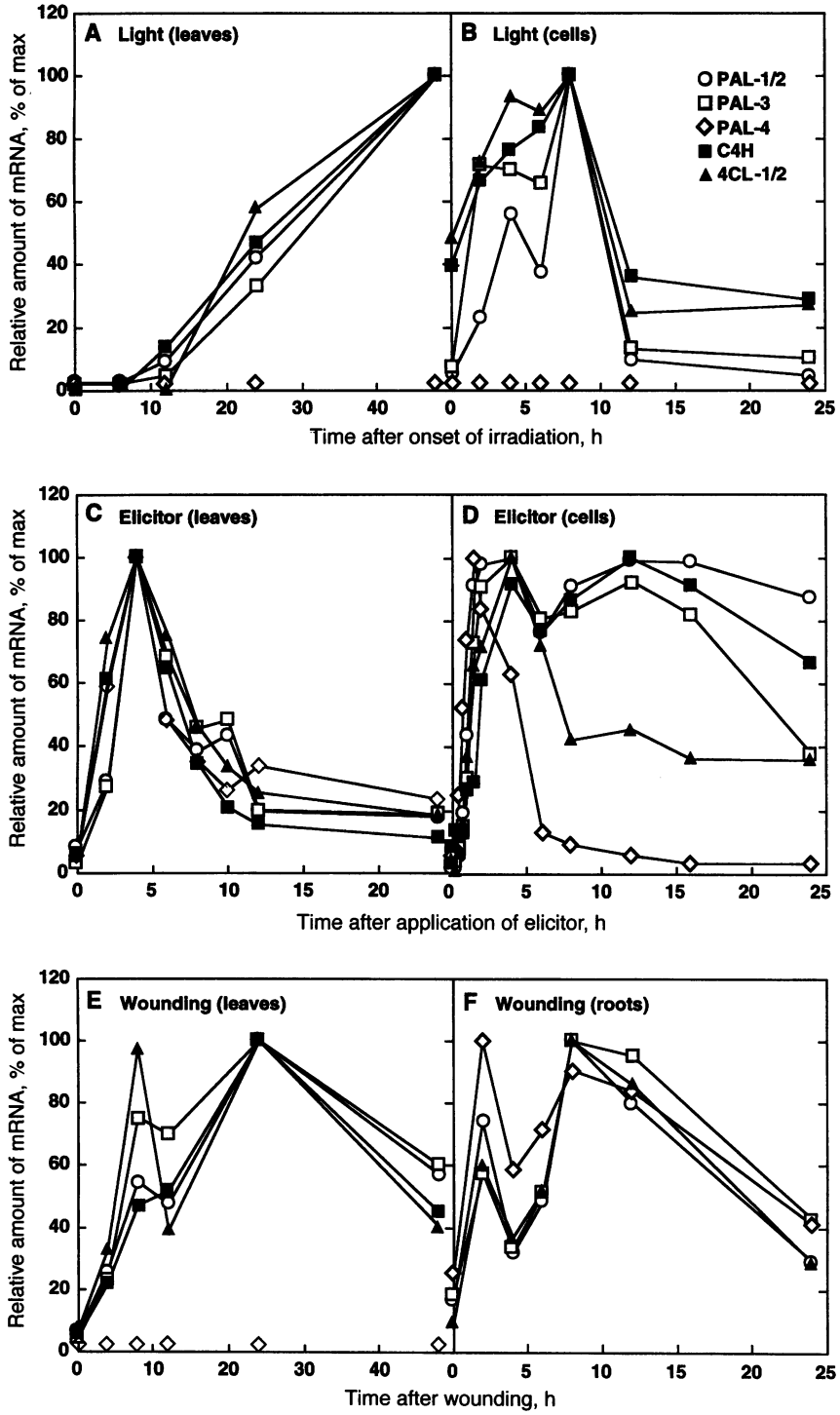

FIG. 2. Timing of induced changes in PAL, C4H, and 4CL mRNAs in differently treated parsley tissues. Samples were taken from leaves, roots, or cultured cells at the indicated times, following irradiation with UV-containing white light, treatment with fungal elicitor, or cutting into ≈2-mm slices (wounding). RNA was extracted, analyzed on Northern blots using the indicated probes, and quantified densitometrically. Note that C4H was not measured in *F*, due to a shortage of RNA.

Fig. 2 shows the induction kinetics for the individual mRNA classes analyzed with these probes in variously treated parsley tissues. Four particularly clear-cut results were obtained: First, UV-containing white light, fungal elicitor, and wounding all strongly induced the transient accumulation of PAL, C4H, and 4CL mRNAs under all conditions tested; second, within experimental error, the sums of all mRNA classes always followed the same time course; third, the induction behavior and the timing of changes in the PAL1/2 and PAL3 mRNA amounts were almost indistinguishable; and fourth, PAL4 mRNA behaved differently under several of the conditions tested. The most remarkable difference between PAL4 and all other PAL mRNAs was the total lack of response to light in both leaves and cultured cells. Upon wounding, PAL4 mRNA was strongly induced in roots but not in leaves, and unlike the other PAL mRNAs, PAL4 mRNA exhibited only a short-lived response to elicitor treatment of cultured cells.

Although the PAL1 and PAL2 mRNAs could not be distinguished by using the 5′ untranslated regions as hybridization probes, separate determination of the two mRNA species was achieved by hybridization with the gene-specific 18-meric oligonucleotide probes described earlier (6, 19). Similar to previous results (19), the timing of induced changes in PAL1 and PAL2 mRNA amounts was essentially the same in several representative cases tested (data not shown).

Promoter Elements. Light or elicitor responsiveness of a particular gene could often be simulated in transient expression assays using transformed parsley protoplasts and appropriate promoter–GUS fusion constructs. Typical examples are promoter fragments from the light-responsive CHS gene (25, 29) or from the elicitor-responsive gene encoding pathogenesis-related protein 2 (PR2; ref. 26). Using these two promoter fragments as well as the empty vector as controls, we tested the three putative cis-acting elements (boxes P, A, and L) from the PAL1 gene promoter, alone and in combinations, by this method. With the exception of the relatively large fragment used as PR2 control, all promoter fragments were analyzed in tetrameric form to ensure high transcriptional efficiency.

The results (Table 2) demonstrate that boxes P and A, whether alone or together, conferred neither constitutive nor inducible expression on the fusion construct. In contrast, the construct containing box L exhibited high basal expression which was, however, not further stimulated by light or elicitor. This level was reduced to background activity upon partial mutation of box L (box Lm, TCTCA*AAGCAA* instead of TCTCACCTACC), a mutation which had previously been shown to strongly impair binding of nuclear proteins (17). The high basal activity level conferred by box L alone was further increased when the entire promoter region containing all three boxes was fused to the reporter gene; but again, light or elicitor responsiveness was not observed. Large effects of light and elicitor on the CHS and PR2 constructs, respectively, confirmed the efficiency of the assay. These data, together with those obtained earlier by *in vivo* DNA footprinting (6), indicate that the PAL- and 4CL-specific boxes P, A, and L, though involved in induced transcriptional activity, are not sufficient to confer responsiveness to UV light or elicitor.

Table 2. Relative expression rates of various promoter fragment–GUS fusion constructs in parsley protoplasts

Promoter fragment*	GUS activity,† pmol of 4-MU/min per mg protein		
	Control	Light	Elicitor
pBT6	12	14	43
4 × CHS^{I+II}	97	4042	66
PR2	33	15	591
4 × P	36	24	37
4 × A	11	13	23
4 × P/A	4	6	23
4 × L	477	337	326
4 × Lm	33	20	37
4 × P/A/L	1098	863	1133

*All PAL promoter elements were derived from the PAL1 gene; P/A and P/A/L additionally contained the sequences between the respective boxes (see Fig. 1).

†Mean values from 6 independent determinations. 4-MU, 4-methylumbelliferone.

DISCUSSION

Copy number reconstructions (6) and extensive screening of genomic and cDNA libraries together provide strong evidence that PAL in parsley is encoded by four genes. Of these four, three (PAL1, PAL2, and PAL3) appear to be regulated very similarly in all parsley organs analyzed (18), as well as in diversely stimulated leaves, roots, and cell cultures (Fig. 2 and ref. 19). The fourth gene, PAL4, exhibited different expression patterns, most notably a total lack of light responsiveness. The strong response of PAL4 to wounding in roots, in sharp contrast to leaves, suggests a tissue-specific component of differential PAL gene expression, in addition to stimulus specificity. Similar conclusions were drawn by others for the bean PAL2 and PAL3 genes, whose promoters, fused to the GUS reporter gene, were differentially expressed in various tissues of transgenic tobacco, potato, and *Arabidopsis* as well as untransformed bean plants (3, 30, 31). However, although these studies enabled a detailed analysis of cell type-specific bean PAL2 and PAL3 expression, they lacked comparison with the third PAL gene (PAL1) that is present in bean (2). Thus, it remains open whether there is similar, apparent redundancy of PAL gene expression in bean or other plant species as suggested by our data for PAL1, PAL2, and PAL3 in parsley.

In parsley, the apparent lack of differential PAL1, PAL2, and PAL3 gene expression is paralleled by an almost total lack of enzyme-kinetic differences among all four PAL isoforms, even including PAL4 (18). This unexplained, apparent redundancy of PAL genes in parsley—and even more likely in tomato with at least 5 PAL genes (14) and potato with 40–50 PAL genes (10)—is particularly surprising in view of the existence of only two of the functionally closely related 4CL genes in both parsley (20) and potato (32). Further investigations at the cellular level in different PAL-active tissues, including subcellular localization studies of the putative multienzyme complex formed by PAL, C4H, and 4CL, will have to clarify the functional relevance of the occurrence of multiple PAL genes. One recent set of experiments in this direction, using *in situ* RNA hybridization, indicated the predominant induction of PAL1/2 mRNA by light in leaf epidermal cells, and of PAL4 mRNA around fungal infection sites in young leaf buds (17).

Whatever the reason for the occurrence of multiple PAL gene copies in parsley and their partially differential regulation, the overall degree of coordination in the regulation of the functionally interdependent genes encoding PAL, C4H, and 4CL is impressive. In contrast to the partially differential behavior within the PAL gene family, the timing of induction and subsequent turnover was always identical, within experimental error, for all three types of functionally related mRNAs, with the possible exception of 4CL mRNA turnover in elicitor-stimulated cell cultures (Fig. 2*D*). This is a rare example of strictly coordinated gene regulation within a set of metabolically related reactions, probably reflecting the fact that, at least in parsley, none of the three enzymes has any known function independent of the other two. This high degree of coordination is further reflected in the occurrence of three common promoter elements, at least in the PAL and 4CL genes. We have now shown that the previously established, inducible *in vivo* DNA footprints on the PAL1 gene

promoter (6) constitute a set of three characteristic sequence motifs (boxes P, A, and L) that are present in all known PAL and 4CL gene promoters. [Three possible exceptions (Table 1) are considered doubtful, because only limited portions of the respective promoters (2, 9, 14) are available for comparison.] Conversely, we have found no example of a gene outside those involved in general phenylpropanoid metabolism, whose promoter contains a complete set of all three boxes, further supporting their functional importance in the coordinate regulation of these genes.

It should be noted that the three boxes were originally identified as sites of *in vivo* (6) and *in vitro* (17, 28) DNA–protein interaction in the PAL1 gene promoter, and their possible function as cis-regulating elements has now been strengthened by sequence comparison of the various PAL and 4CL genes. Thus, assignment of the individual boxes in all other PAL and 4CL gene promoters (Table 1) is based solely on sequence similarity with the more rigorously defined parsley PAL1 boxes. Under these conditions, we notice a large similarity between major parts of the box P and box L consensus sequences, particularly between the central part of box P and the 3′ part of box L (e.g., ACACCAACC in the various 4CL gene promoters), and do not exclude a functional overlap. Furthermore, the distances and relative positions of the individual boxes seem to be rather variable within the TATA-proximal promoter region and may, together with some deviation from the consensus sequence, have a bearing on the particular mode of gene expression. This latter possibility is suggested by the apparent correlation between promoter architecture and expression behavior among the four PAL genes in parsley. While PAL1, PAL2, and PAL3, exhibiting similar arrangements of the three boxes, are expressed similarly in various parsley organs (18) as well as upon exogenous stimulation (Fig. 2), PAL4 differs considerably with respect to both promoter architecture and expression behavior. However, the precise contributions of the three boxes to particular modes of expression have yet to be established.

In any event, our present results indicate that the three boxes alone are insufficient for PAL gene activation by light or elicitor, although their functional relevance for transcriptional regulation by these two stimuli has been demonstrated in several different ways: by *in vivo* DNA footprinting (6), by gel-mobility-shift assays using box P and box L probes and nuclear protein extracts (17, 28), and by specific binding of a putative transcription factor to box P (28). We therefore assume that one or more additional cis-acting elements are involved in light and elicitor responsiveness of the PAL genes in parsley. Such an element(s) may be located either upstream of box P or downstream of the transcription start site, or both. A location downstream of the transcription start site is particularly likely in view of recent results in this direction obtained with the parsley 4CL1 gene (21). Analogous studies of the PAL1 gene, using transgenic parsley plants, should be informative.

Considering the high degree of coordinate regulation of PAL, C4H, and 4CL on the one hand, and the occurrence of boxes P, A, and L on all PAL and 4CL gene promoters on the other, it is tempting to speculate that this set of promoter elements will also be found on the C4H gene promoter, in parsley and probably in other plant species as well, and that the apparent tight coregulation of the PAL, C4H, and 4CL genes is due, at least in part, to the combined activities of the observed common and unique set of three cis-acting elements and their cognate trans-acting factors. The partially differential behavior of the PAL4 gene suggests a remarkable degree of variability within these combinatorial effects and that deviations from element consensus sequences and variations in relative element positions may have significant roles in this regard.

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1. Koukol, J. & Conn, E. E. (1961) *J. Biol. Chem.* **236,** 2692–2698.
2. Cramer, L. C., Edwards, K., Dron, M., Liang, X., Dildine, S. L., Bolwell, G. P., Dixon, R. A., Lamb, C. J. & Schuch, W. (1989) *Plant Mol. Biol.* **12,** 367–383.
3. Liang, X., Dron, M., Cramer, C. L., Dixon, R. A. & Lamb, C. J. (1989) *J. Biol. Chem.* **264,** 14486–14492.
4. Liang, X., Dron, M., Schmid, J., Dixon, R. A. & Lamb, C. J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9284–9288.
5. Minami, E., Ozeki, Y., Matsuoka, M., Koizuka, N. & Tanaka, Y. (1989) *Eur. J. Biochem.* **185,** 19–25.
6. Lois, R., Dietrich, A., Hahlbrock, K. & Schulz, W. (1989) *EMBO J.* **8,** 1641–1648.
7. Schulz, W., Eiben, H. G. & Hahlbrock, K. (1989) *FEBS Lett.* **258,** 335–338.
8. Ohl, S., Hedrick, S. A., Chory, J. & Lamb, C. J. (1990) *Plant Cell* **2,** 837–848.
9. Warner, L. A., Li, G., Ware, D., Somssich, I. E. & Davis, K. R. (1995) *Plant Mol. Biol.* **27,** 327–338.
10. Joos, H. J. & Hahlbrock, K. (1992) *Eur. J. Biochem.* **204,** 621–629.
11. Whetten, R. W. & Sederoff, R. R. (1992) *Plant Physiol.* **98,** 380–386.
12. Yamada, T., Tanaka, Y., Sriprasertsak, P., Kato, H., Hashimoto, T., Kawamata, S., Ichinose, Y., Kato, H., Shiraishi, T. & Oku, H. (1992) *Plant Cell Physiol.* **33,** 715–725.
13. Yamada, T., Sriprasertsak, P., Kato, H., Hashimoto, T., Shimizu, H. & Shiraishi, T. (1994) *Plant Cell Physiol.* **35,** 917–926.
14. Lee, S. W., Robb, J. & Nazar, R. N. (1992) *J. Biol. Chem.* **267,** 11824–11830.
15. Subramaniam, R., Reinold, S., Molitor, E. K. & Douglas, C. J. (1993) *Plant Physiol.* **102,** 71–83.
16. Hahlbrock, K. & Scheel, D. (1989) *Annu. Rev. Plant Physiol. Mol. Biol.* **40,** 347–369.
17. Hahlbrock, K., Scheel, D., Logemann, E., Nürnberger, T., Parniske, M., Reinold, S., Sacks, W. R. & Schmelzer, E. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4150–4157.
18. Appert, C., Logemann, E., Hahlbrock, K., Schmid, J. & Amrhein, N. (1994) *Eur. J. Biochem.* **225,** 491–499.
19. Lois, R. & Hahlbrock, K. (1992) *Z. Naturforsch. C. Biosci.* **47,** 90–94.
20. Douglas, C., Hoffmann, H., Schulz, W. & Hahlbrock, K. (1987) *EMBO J.* **6,** 1189–1195.
21. Douglas, C. J., Hauffe, K. D., Ites-Morales, M. E., Ellard, M., Paszkowski, U., Hahlbrock, K. & Dangl, J. L. (1991) *EMBO J.* **10,** 1767–1775.
22. Hauffe, K. D., Paszkowski, U., Schulze-Lefert, P., Hahlbrock, K., Dangl, J. L. & Douglas, C. J. (1991) *Plant Cell* **3,** 435–443.
23. Hauffe, K. D., Lee, P. S., Subramaniam, R. & Douglas, C. J. (1993) *Plant J.* **4,** 235–253.
24. Weisshaar, B., Armstrong, G. A., Block, A., da Costa e Silva, O. & Hahlbrock, K. (1991) *EMBO J.* **10,** 1777–1786.
25. Schulze-Lefert, P., Dangl, J. L., Becker-Andre, M., Hahlbrock, K. & Schulz, W. (1989) *EMBO J.* **8,** 651–656.
26. van de Löcht, U., Meier, I., Hahlbrock, K. & Somssich, I. E. (1990) *EMBO J.* **9,** 2945–2950.
27. Mizutani, M., Ward, E., DiMaio, J., Ohta, D., Ryals, J. & Sato, R. (1993) *Biochem. Biophys. Res. Commun.* **190,** 875–880.
28. Da Costa e Silva, O., Klein, L., Schmelzer, E., Trezzini, G. F. & Hahlbrock, K. (1993) *Plant J.* **4,** 125–135.
29. Schulze-Lefert, P., Becker-André, M., Schulz, W., Hahlbrock, K. & Dangl, J. L. (1989) *Plant Cell* **1,** 707–714.
30. Leyva, A., Liang, X., Pintor-Toro, J. A., Dixon, R. A. & Lamb, C. J. (1992) *Plant Cell* **4,** 263–271.
31. Shufflebottom, D., Edwards, K., Schuch, W. & Bevan, M. (1993) *Plant J.* **3,** 835–845.
32. Becker-André, M., Schulze-Lefert, P. & Hahlbrock, K. (1991) *J. Biol. Chem.* **266,** 8551–8559.