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Communication

Light and Fungal Elicitor Induce 3-Deoxy-D-*arabino*-Heptulosonate 7-Phosphate Synthase mRNA in Suspension Cultured Cells of Parsley (*Petroselinum crispum* L.)[1]

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ABSTRACT

Light and fungal elicitor induce mRNA encoding 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase in suspension cultured cells of parsley (*Petroselinum crispum* L.). The kinetics and dose response of mRNA accumulation were similar for DAHP synthase and phenylalanine ammonia-lyase (PAL). Six micrograms of elicitor from *Phytophthora megasperma* f. *glycinia* gave a detectable induction within 1 hour. Induction of DAHP synthase and PAL mRNAs by light was transient, reaching maximal levels at 4 hours and returning to pretreatment levels after 24 hours. Our data suggest that either light or fungal elicitor transcriptionally activate DAHP synthase. A coordinate regulation for key enzymes in the synthesis of primary and secondary metabolites is indicated.

The shikimate pathway is common to the synthesis of the three aromatic amino acids: phenylalanine, tryptophan, and tyrosine. These amino acids serve dual purposes as substrates in protein synthesis and the synthesis of secondary products like lignin, suberin, phytoalexins, and UV light protective compounds. The first enzyme of the shikimate pathway, DAHP[3] synthase, is regulated developmentally in carrot suspension cells (21) or tomato pericarp (11) and by wounding (5) or glyphosate treatment (19) of Solanaceae. Wounding induces a transient accumulation of DAHP synthase mRNA (5) in potato tuber. Maximal expression is seen within 6 h. Similar induction kinetics for PAL mRNA suggest a coordinated pattern of expression for enzymes of primary and secondary aromatic metabolic pathways (5).

Phenylpropanoid biosynthesis in parsley suspension cultures responds to both UV light and fungal elicitor treatment (9). Flavonoids and furanocoumarin phytoalexins are both made from phenylalanine, by two different routes (8). Branch pathway-specific and core enzymes of phenylpropanoid metabolism are coordinately regulated at the transcriptional level by fungal elicitation or by UV light (2). PAL catalyzes the first committed step of phenylpropanoid metabolism, and is therefore common to the biosynthesis of all phenylpropanoid-derived products. The enzyme is encoded by at least four genes, three of which are induced by both elicitor and UV light (3).

The integration of aromatic amino acid biosynthesis with production of secondary metabolites derived from these amino acids has not been studied in much detail. Recently, a 2.0- and 1.6-fold increase of DAHP synthase was shown in parsley cell cultures treated with elicitor (16) or light (17), respectively. In this paper, we characterize DAHP synthase mRNA induction in response to these treatments. The rapid induction of DAHP synthase mRNA and the kinetics and magnitude of the induction are similar to the PAL mRNA response. These data suggest a coordination of secondary metabolite biosynthesis with primary metabolism at the transcriptional level.

MATERIALS AND METHODS

Cell Cultures

Suspension cultured cells of parsley (*Petroselinum crispum* [Mill] Nyman ex A.W. Hill [*P. hortense* Hoffm.]) were a gift of Joe Chappell (University of Kentucky, Lexington), and were grown in the dark as described previously (16).

Elicitor Treatment

To ensure homogeneity for elicitor treatments, several 9-d-old cell cultures were mixed together, and 25 mL aliquots were transferred to 125 mL Erlenmeyer flasks immediately prior to the induction treatment or the addition of inhibitor. Elicitor was prepared from lyophilized mycelia of *Phytophthora megasperma* f. *glycinia*, a gift from D. Kuhn (Purdue University, West Lafayette, IN), as described (1). Actinomycin D was added to cultures from a stock solution of 1.67 mg/mL in 95% ethanol, to give a final concentration of 20 μg/mL.

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[3] Abbreviations: DAHP, 3-deoxy-D-*arabino*-heptulosonate 7 phosphate; PAL, phenylalanine ammonia-lyase.

Light Induction

For light induction, several 9-d-old cultures were mixed together and aliquoted as above. Cultures were placed beneath fluorescent lights as described previously (17).

Nucleic Acid Analysis

Total RNA was isolated from suspension cultured cells that had been harvested by filtration and frozen in liquid nitrogen (10). Northern blots were performed as described previously (10). For each experiment, 10 μg of total RNA was separated on 1.2% agarose gels containing 7.5% formaldehyde. The RNA was transferred onto nitrocellulose filters (Schleicher and Schuell, BA85 NC) and fixed by UV irradiation (Stratalinker, Stratagene, Inc.). mRNAs were detected with random primed ^{32}P-labeled heterologous DNA sequences encoding DAHP synthase and PAL under the following conditions (14): hybridization was at 55°C in 6 × SSC containing 0.1% SDS, 5 × Denhardt's solution, and 10 mg/L boiled herring sperm DNA. Washing was at 55°C in 3 × SSC containing 0.1% SDS. 1 × SSC is 15 mM sodium citrate, 150 mM NaCl, pH 7; Denhardt's solution is 0.02% each of Ficoll, BSA, and PVP (mol wt 40,000). The probe used to detect parsley DAHP synthase mRNA was the 356 base pair *Kpn*I/*Eco*RI fragment of the potato cDNA (6). The PAL probe pP10Ss3.3 was a *Glycine max* genomic DNA fragment and a gift from Dr. Lila Vodkin (University of Illinois, Champaign-Urbana).

RESULTS AND DISCUSSION

Elicitor from *P. megasperma* f. *glycinia* causes a rapid accumulation of DAHP synthase mRNA in parsley cells grown in suspension cultures (Fig. 1A, B). DAHP synthase mRNA accumulation is dependent upon both the concentration of elicitor (Fig. 1A) and the time after treatment (Fig. 1B). Maximum accumulation of DAHP synthase mRNA was observed using 60 to 90 μg/mL elicitor, with a detectable response in our system at 6 μg/mL elicitor. Actinomycin D, an inhibitor of transcription in eukaryotic cells, caused a significant decrease in DAHP synthase mRNA accumulation (Fig. 1A). Elicitor-induced accumulation of DAHP synthase mRNA reached maximal levels within 2 h (Fig. 1B) and remained elevated for at least 24 h after treatment with 60 μg/mL elicitor (data not shown).

A comparison of both the time course and the dose response of DAHP synthase and PAL mRNA accumulation in elicitor-treated parsley cells suggests that these two enzymes are coordinately expressed. The expression patterns for both enzymes show a similar increased rate of accumulation between 45 and 60 min after treatment with elicitor (Fig. 1B).

Treatment of parsley cells with light induces a transient accumulation of DAHP synthase mRNA (Fig. 1C, D). This accumulation begins within 2 h of light treatment and continues up to 8 h (Fig. 1C). After 24 h, levels of DAHP synthase mRNA have returned to pretreatment levels (Fig. 1D), contrasting the sustained expression by elicitor.

Similarities between the kinetics of induction of DAHP synthase mRNA and PAL mRNA accumulation in response to elicitor and light treatments could indicate that DAHP synthase and PAL genes are responding to the same signal(s).

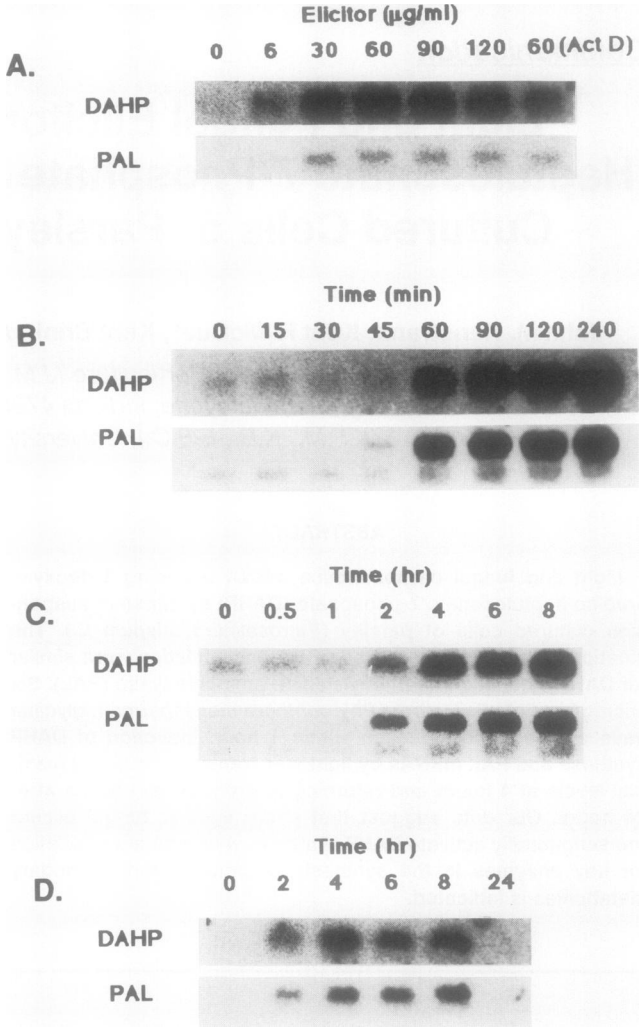

Figure 1. DAHP synthase and PAL mRNAs in parsley cell suspension cultures in response to elicitor from *P. megasperma* and light. A, Elicitor dose response. Cells were treated with the indicated amounts of elicitor or with 60 μg mL^{-1} elicitor plus 20 μg mL^{-1} actinomycin D and harvested 6 h after treatment. B, Kinetics of elicitor induction. Cells were treated with 60 μg mL^{-1} elicitor and incubated for the indicated times. C and D, Time course of light induction. Separate experiments with different time frames emphasize early (C) and extended (D) exposure to UV light. Blots were probed with cDNAs encoding potato DAHP synthase or *G. max* PAL.

On the other hand, it is also possible that DAHP synthase transcription is responding to a depletion of phenylalanine pools caused by increased PAL enzyme activity. Small but significant increases in PAL enzyme activity are observed within 1 h in response to elicitor treatment, reaching a 10-fold maximum in parsley cell suspension cultures after 8 h (12, 16). The small change in PAL activity, even after 1 h, may have a significant effect upon the phenylalanine pool size, thereby triggering DAHP synthase mRNA induction.

Transcriptional inhibition by feedback regulatory mechanisms has recently been observed in plants. Ethylene inhibits transcription of amino cyclopropane carboxylate synthase in wounded *Cucurbita* tissue (18) and *trans*-cinnamate inhibits

elicitor-caused induction of PAL in suspension cultures of French bean (15). Nuclear run-on analysis is required to differentiate between various regulatory transcriptional mechanisms for plant DAHP synthase.

PAL has been studied extensively at the enzyme and genetic levels (3, 9). Changes in phosphorylation (4) and the involvement of protein kinase-mediated signal transduction have been implicated in gene activation of PAL (7). In parsley, *cis*-acting elements have been identified that appear to participate in transcriptional activation by fungal elicitation and by UV light (13). DNA sequences within these elements are conserved in the promoters of at least one PAL gene (*PAL-1*) and the coordinately regulated chalcone synthase gene of parsley. Consensus DNA sequences have been identified for several light-regulated promoters (20). We found a homologous region within a potato DAHP synthase promoter that shows 65% identity (our manuscript in preparation) with the light responsive element of parsley. Thus, DAHP synthase may interact with the same signal transduction pathway. The elucidation of mechanisms of feedback and coordinate regulation with respect to secondary product synthesis is a goal of future study. DAHP synthase gene expression also concerns normal developmental control and constitutive cues that are quite distinct from those related to plant defense. Thus, the enzyme's regulatory properties will certainly reflect its complex role in plant metabolism.

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