Geranyl Diphosphate: 4-Hydroxybenzoate Geranyltransferase from Lithospermum erythrorhizon

CLONING AND CHARACTERIZATION OF A KEY ENZYME IN SHIKONIN BIOSYNTHESIS*

Received for publication, July 9, 2001, and in revised form, December 10, 2001 Published, JBC Papers in Press, December 14, 2001, DOI 10.1074/jbc.M106387200

Kazufumi Yazaki‡, Miyuki Kunihisa, Takahiro Fujisaki, and Fumihiko Sato

From the Molecular & Cellular Biology of Totipotency, Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kitashirakawa, Kyoto 606-8502, Japan

Two cDNAs encoding geranyl diphosphate:4-hydroxybenzoate 3-geranyltransferase were isolated from Lithospermum erythrorhizon by nested PCR using the conserved amino acid sequences among polyprenyltransferases for ubiquinone biosynthesis. They were functionally expressed in yeast COQ2 disruptant and showed a strict substrate specificity for geranyl diphosphate as the prenyl donor, in contrast to ubiquinone biosynthetic enzymes, suggesting that they are involved in the biosynthesis of shikonin, a naphtoquinone secondary metabolite. Regulation of their expression by various culture conditions coincided with that of geranyltransferase activity and the secondary metabolites biosynthesized via this enzyme. This is the first established plant prenyltransferase that transfers the prenyl chain to an aromatic substrate.

The prenylation reaction of aromatic substrates is involved in the biosynthesis of diverse molecules that play important roles in various biological activities in bacteria to mammals, e.g. electron transport via ubiquinone and plastoquinone (1). In higher plants, such prenylation largely contributes to the diversification of aromatic secondary metabolites, with regard to both their chemical structures and biological activities. For instance, some prenylated flavonoids act as phytoalexins that are involved in plant defense mechanisms (2, 3), and have also been reported to be potential natural medicines (4–6). Due to these interesting properties, intensive biochemical studies have been conducted to identify plant prenyltransferases which catalyze the transfer of prenyl diphosphate to an aromatic ring to generate a prenylated derivative, such as flavones (7, 8), isoflavonones (9–12), coumarins (13, 14), olivetolate (15), and 4-hydroxybenzoate (16). However, there has been no report on the identification of the genes that encode such prenyltransferases involved in the biosynthesis of these secondary metabolites.

Shikonin, a red naphtoquinone derivative, is a secondary metabolite that specifically occurs in boraginaceous plants, and is the active principle of the medicinal plant Lithospermum erythrorhizon (17). Since this compound and its derivatives exhibit antibacterial activity, their functions as phytoalexins have also been reported (18). The biosynthesis of shikonin includes a key prenylation step catalyzed by geranyl diphosphate (GPP)1:4-hydroxybenzoate (4HB) 3-geranyltransferase; i.e. coupling of the shikimate and mevalonate pathways (17, 19, 20). This enzyme plays a critical role in the regulation of shikonin biosynthesis in cell cultures of L. erythrorhizon, i.e. up- and down-regulation of this enzyme activity directly affects the production of shikonin (21, 22). The enzyme activity is strongly suppressed by light irradiation, ammonium ion, and the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) (23), whereas this activity is enhanced with the addition of oligogalacturonide (OG) (24) and methyl jasmonate (MJ) (25) to the medium. It has also been reported using a partially purified enzyme that this protein is ER membrane-bound and shows strict substrate specificity for geranyl (C10) diphosphate for the chain length of the prenyl donor (16), which is in clear contrast to the mitochondrial polyprenyltransferase for ubiquinone biosynthesis (26). We attempted to isolate the cDNA of geranyltransferase from L. erythrorhizon as a model, since these characteristic properties should be advantageous for identifying the gene product.

In this report, we describe the isolation of two cDNAs for the prenyltransferase, designated LePGT-1 and -2 (L. erythrorhizon p-hydroxybenzoate:geranyltransferase) from L. erythrorhizon cultured cells, and functional expression of the gene products in yeast. A direct correlation between this gene expression and shikonin accumulation shows that they are involved in the secondary metabolism.

EXPERIMENTAL PROCEDURES

Plant Material, Reagents—Cell suspension cultures (strain M18) (27) were maintained in Linsmaier and Skoogs (LS) medium (28). They produced shikonin derivatives when cultured in M9 medium (29) in darkness (20–23). In the present study, 4.5 ml of liquid paraffin was placed over 30 ml of M9 medium to extract shikonin derivatives produced by the cells (24), which interfere with the measurement of enzyme activity. GPP and dimethylallyl diphosphate were synthesized as previously described (30). Other chemical reagents were purchased from Wako Pure Chemicals (Japan), Nacalai (Japan), and Sigma.

Nested PCR and Rapid Amplification of cDNA Ends—A double-stranded cDNA library with an adapter sequence at both ends was synthesized from poly(A)+ RNA, which was prepared from the cells cultured in M9 medium for 4 days in the dark, with Marathon cDNA Amplification Kit (CLONTECH). In the first PCR, AP-1 from the kit and a degenerate primer (Rv-1) designed from the conserved sequences

† To whom correspondence should be addressed: Molecular & Cellular Biology of Totipotency, Div. of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kitashirakawa, Kyoto 606-8502, Japan. Tel.: 81-75-753-6384; Fax: 81-75-753-6398; E-mail: yazaki@kais.kyoto-u.ac.jp.

‡ To whom correspondence should be addressed: Molecular & Cellular Biology of Totipotency, Div. of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kitashirakawa, Kyoto 606-8502, Japan.

* This work was supported in part by a grant-in-aid for Scientific Research from the Japanese Society for Promotion of Science (to K. Y.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: GPP, geranyl diphosphate; 4HB, 4-hydroxybenzoate; 2,4-D, 2,4-dichlorophenoxyacetic acid; OG, oligogalacturonide; MJ, methyl jasmonate; PGT, p-hydroxybenzoate:geranyltransferase; LS, Linsmaier and Skoog; ER, endoplasmic reticulum; 4HPT, 4HB:hexaprenyltransferase; PMA1, plasma membrane ATPase 1.

This paper is available on line at http://www.jbc.org

6240 This paper is available on line at http://www.jbc.org
among coq2 and its orthologs were used as forward and reverse primers, respectively. The latter sequence, 5′-GGCGTDSWVTVTACCC-3′, corresponded to the amino acid sequence n-GKSTA-ε. The reaction mixture (0.3 µl) was further used as the template for the second PCR, using AP-2 from the kit and another degenerate nucleotide (Rv-2), 5′-TCYTG- 
GRTGDGCRTADATDGTRTC-3′. The products were amplified in Tris-HCl buffer (pH 7.5) containing 0.6 M sorbitol, 1 mM phenylmethylsulfonyl fluoride, and 10 mM dithiothreitol, the mixture was centrifuged at 10,000 
RF1402 (0.3 
H9262, with the paraffin layer. The amounts of these secondary metabolites in paraffin were significantly similar to yeast 4HPT and isolated and classified into two groups. Full-length clones corresponding to both LePGT-1 and -2, were used as probes for the specific detection of each molecular species. The β-subunit of ATP-synthase was used as a control (36).

Inhibitors of geranyltransferase expression, NH4Cl (1 mM) and 2,4-D (1 µM), were added to M9 medium and cells were harvested 7 days after incubation. After solvents as specified above, the microsomal fraction was resuspended in 10 mM Tris-HCl buffer (pH 7.5) containing 0.6 M sorbitol, 1 mM phenylmethylsulfonyl fluoride, and 10 mM dithiothreitol, the mixture was centrifuged at 10,000 
GPP:4HB Geranyltransferase in Shikonin Biosynthesis

Cloning and Sequence Analysis of LePGT cDNAs—We attempted to isolate a cDNA encoding 4HB:geranyltransferase which is involved in the secondary metabolism of L. erythrorhizon by a molecular biological approach, since purification of this membrane-bound enzyme to homogeneity was unsuccessful (16). The enzymatic reaction of the geranyltransferase is functionally analogous to that of the prenyltransferase in ubiquinone biosynthesis, i.e. 4HB:polyisoprenyltransferase (Fig. 1A). Three genes for such prenyltransferases that accept 4HB and polyisoprenyl diphosphate as a prenyl acceptor and donor have been reported; 4HPT is the gene product of COQ2 (yeast) (26), 4HB:polyisoprenyl diphosphate transferase (ppt1) of fission yeast (38), and 4HB:octaprenyltransferase (the gene product of ubikA) of Escherichia coli (39). Based on multiple alignment with these polyisoprenyltransferases and other eukaryotic orthologs in protein data bases, two degenerate PCR primers were designed from the conserved amino acid sequences. A library of cDNAs with adopter sequences was prepared from L. erythrorhizon cells cultured in M9 medium in the dark, in which the geranyltransferase was highly expressed. By nested PCR, a smear band was amplified, which was then subcloned and randomly sequenced. Nine DNA fragments that were significantly similar to yeast 4HPT were isolated and classified into two groups. Full-length clones corresponding to both groups were isolated by 3′-rapid amplification of cDNA ends, and were designated LePGT-1 and -2.

LePGT-1 (1,153 nucleotides) encodes an open reading frame corresponding to a protein of 307 amino acids, whereas LePGT-2 (1,240 nucleotides) has an open reading frame for 306 amino acids. The sequence identity between them was 76 and 93% on a nucleotide and amino acid basis, respectively. The amino acid sequence similarity to yeast 4HPT was only 35% for the full-length and 53% for the conserved region.

A multiple alignment among eukaryotic orthologs in the data bases is shown in Fig. 1B. Both LePGT clones possess the motif NDXXD for putative prenyl diphosphate binding (40), and a GX(K/Y)STAL sequence which seemed to be specifically conserved in this subfamily of 4HB:prenyltransferase, although the function of this domain is not yet known (Fig. 1B). Another highly conserved sequence, YDTYAIQFD, is observed in the alignments with yeast coq2 and plant equivalent members. The polypeptide sequence of Arabidopsis has an extended leader peptide, which may be a mitochondrial signal according to the TargetP and ChloroP programs on CBS servers (www.cbs.dtu.dk/). LePGT-1 and -2 do not possess such a signal peptide for mitochondrial sorting, but are presumed to be sorted to the ER (41), which coincided the subcellular local-
Fig. 1. A, enzymatic reaction of two prenyltransferases. LePGT is a specific prenyltransferase for geranyl diphosphate leading to shikonin or dihydroechinofuran in *L. erythrorhizon*, whereas the gene product of *COQ2*, hexaprenyltransferase, is responsible for ubiquinone biosynthesis in yeast. G-4HB, geranyl-4-HB; HP-4HB, hexaprenyl-4HB; HPP, hexaprenyl diphosphate. B, multiple alignment of eukaryotic orthologs of 4HB:prenyltransferases. Ce, *C. elegans* (U13876-5); At, *Arabidopsis thaliana* (AC004521-32); Sp, *Schizosaccharomyces pombe* (Z68728-4); Sc, *Saccharomyces cerevisiae* (M16989, *COQ2*). The putative prenyl diphosphate binding motif NDXX and the GX(K/Y)STAL motif are highlighted with solid underlines. Annealing sites of degenerate primers (Rv-1 and -2) and a specific 3′ rapid amplification of cDNA ends primer (Fw-1) are indicated with arrows below the sequences. Potential transmembrane domains for LePGTs are depicted by hatched bars above the corresponding regions of the protein.

Functional Expression of LePGTs in a Yeast *COQ2* Disruptant—To demonstrate the enzymatic activity of LePGT gene products, yeast was used for heterologous expression because of their high hydrophobicity. Wild type yeast (strain W303-1A), however, possesses 4HPT for ubiquinone biosynthesis localized in the mitochondria, which may raise the background level in the control experiment because of its broad substrate specificity for prenyl donor (43). Thus, the gene *COQ2* was disrupted according to the method of Güldener et al. (31), and the disruptant W303Δcoq2, which is unable to use the nonfermentable carbon source glycerol, was used as a heterologous host to express LePGT cDNAs (Fig. 2). The growth defect of W303Δcoq2 on glycerol plate was complemented by reintroduction of *COQ2* gene subcloned into a shuttle vector, pDR196, which had a PMA1 promoter to drive the heterologous gene in yeast cells. Full-length LePGT-1 and -2 subcloned into pDR196 were also introduced into W303Δcoq2, but they did not complement the growth on the glycerol medium.

The enzymatic activities of 4HB:prenyltransferase was measured with these yeast transformants. As expected, the wild type yeast showed apparent 4HB:geranyltransferase activity in the mitochondrial fraction due to the *COQ2* gene (Fig.
3A), but the same fraction of disruptant did not show this enzyme activity. W303Δcoq2 transformed with full-length LePGT-1 and -2 were grown in YPAD medium and the enzyme activity was measured. The empty vector was used as a negative control. As depicted in Fig. 3A, both LePGT-1 and -2 showed an apparent activity of 4HB:geranyltransferase in the microsomal fraction using 4HB and GPP as the substrates, whereas the transformant of the empty vector gave almost no detectable activity. The activities of marker enzymes for mitochondria (cytochrome c oxidase) and ER (NADPH-cytochrome c reductase) in these fractions are also shown. Although the mitochondrial fraction contains ER membrane, the contamination of mitochondria in the microsomal fraction is very low. Geranyltransferase activity was undetectable in the supernatant (centrifugation at 100,000 × g) of these two LePGT transformants. Using the method of Lineweaver and Burk (44), the Km values of recombinant LePGT-1 and -2 for 4HB were determined as 10.3 and 53.8 μM, whereas those for GPP were 5.1 and 45.9 μM, respectively.

The most important criterion for determining whether the LePGTs are involved in shikonin biosynthesis is substrate specificity for the prenyl donor, since the geranyltransferase in the biosynthesis of shikonin and dihydroechinofuran is exclusively specific for GPP, whereas 4HPT for ubiquinone shows a broad substrate specificity regarding the chain length of prenyl diphosphate. Fig. 3B shows the substrate specificity of recombinant proteins of LePGT-1 and -2 in comparison with that of native mitochondrial 4HPT of wild type yeast. The yeast 4HPT has a wide preference for prenyl diphosphate of different chain lengths, e.g. it showed higher activity with geranylgeranyl diphosphate than with GPP as the substrate. In contrast, both gene products derived from L. erythrorhizon could accept only GPP and not dimethylallyl diphosphate, farnesyl diphosphate, or geranylgeranyl diphosphate as the substrate. This clear substrate specificity indicates that both LePGT-1 and LePGT-2 encode the geranyltransferases for secondary metabolism in this plant.

Expression of LePGT Genes in L. erythrorhizon—The activity of 4HB:geranyltransferase in L. erythrorhizon is dramatically induced when the cells are cultured in M9 medium in the dark, but not under light irradiation (21). In accordance with this enzyme activity, Northern analyses showed that both mRNAs were strongly induced in the dark whereas they were dramatically suppressed under illumination (Fig. 4A). These mRNA levels relative to those at day 0 are also shown in Fig. 4B, and are consistent with the accumulation pattern of shikonin derivatives in M9 medium (Fig. 4C).

Geranyltransferase also plays a critical role in the chemical regulation of shikonin production, i.e. this activity is up-regulated with MJ (25) as well as OG (24), whereas its expression is down-regulated by NH4+ and the auxin, 2,4-D (23). The effects of these chemical regulators on LePGT gene expression were analyzed by Northern hybridization. The effects of MJ and OG were investigated in LS medium in which geranyltransferase activity remained very low, but with the addition of both regulators, the LePGT mRNA levels clearly increased (Fig. 5, A and B, left) along with the enzyme activity (Fig. 5C, left). The increase in geranyltransferase activity reflected the accumulation of dihydroechinofuran, which is quickly biosynthesized from the enzymatic reaction product, 3,4-dihydroxybenzoic acid (Fig. 1A), and is therefore a more suitable metabolite than shikonin for monitoring the effect of geranyltransferase induction (24, 45) (Fig. 5D, left). The effects of inhibitors, which were observed in M9 medium, were also consistent with the mRNA level, enzyme activity, and secondary product accumulation, i.e. the accumulation of both LePGT mRNAs as well as the enzyme activity apparently decreased with NH4+ or 2,4-D, along with the dihydroechinofuran level (Fig. 5, right panels).

In the intact plant, shikonin is solely accumulated in underground parts (34), since its biosynthesis is completely inhibited.

![Image](http://www.jbc.org/doi/abs/10.1074/jbc.624303215?high wire=true)
under light conditions. The mRNAs of both LePGT genes were undetectable in aerial plant tissues, and were exclusively detected in root tissues, similar to shikonin accumulation, as shown in Fig. 6. Along with the strict substrate preference for GPP mentioned above, these expression studies support the notion that both cDNAs encode the geranyltransferases involved in shikonin biosynthesis.

DISCUSSION

In this study, we cloned and performed structural analyses of two cDNAs that encode geranyl diphosphate:4-hydroxybenzoate 3-geranyltransferase from cultured _L. erythrorhizon_ cells (Fig. 1). Heterologous expression in yeast showed that both gene products exhibit enzyme activity to transfer the geranyl moiety to an aromatic substrate 4HB, and in particular they show exclusive specificity for the C_{10} substrate GPP (Fig. 3), which are critical points for determining that these enzymes are responsible for shikonin biosynthesis (16).

Each organism has a specific chain length for prenyl moiety of ubiquinone molecule, and the side chain is usually penta- to decaprenyl residue, i.e. ubiquinone-5 to -10. It is very unlikely that the diprenyl-4HB, which is the specific product of LePGTs, is converted to ubiquinone having geranyl residue as the prenyl chain. To exclude the possibility that LePGTs might be involved in ubiquinone biosynthesis in vivo, complementation studies were also done. The yeast _COQ2_ disruptant was transformed with LePGT-1 or -2, but the transformants failed to grow on glycerol medium, whereas the _COQ2_-containing plasmid, as a positive control, complemented the phenotype of disruptant (Fig. 2). Similarly, the plant ortholog _AtPPT-1_ also showed the same complementation in _COQ2_-disrupted fission cell cultures to which the indicated regulators were added. _D_, the production level of dihydroechinofuran in response to addition of a regulator of geranyltransferase.

**FIG. 4.** A, Northern blot hybridization of LePGT-1 and LePGT-2. Their mRNAs were detected with each sequence-specific probe. Cultured cells were agitated either in the dark or under continuous white light in M9 medium. ATP synthase β-subunit was used as the load control. _B_, relative mRNA levels of LePGT-1 and LePGT-2 are normalized using the ATP synthase β-subunit expression level as the internal standard. The relative mRNA level is calculated with the level at day 0 as one. _C_, time course of shikonin productivity either in darkness or under continuous illumination.

**FIG. 5.** Effects of inducers and inhibitors of shikonin production on the expression level of LePGT-1 and -2, as well as geranyltransferase activity and secondary metabolite production. _A_, Northern blot hybridization of LePGT-1 and LePGT-2 in the presence of regulatory elements. _LS_, Linsmaier-Skoog liquid medium; _cn_, control; _MJ_, 10 μM; _OG_, 100 μg/ml; _NH_4Cl (1 mM); 2,4-D, 1 μm. rRNA was used as the load control. _B_, relative mRNA levels of LePGT-1 and LePGT-2 are shown normalized by the rRNA level and expressed with the level at day 0 as one. _C_, enzyme activity of 4HB:geranyltransferase in cell cultures to which the indicated regulators were added. _D_, the production level of dihydroechinofuran in response to addition of a regulator of geranyltransferase.
yeast. These results strongly support our conclusion that the LePGT cDNAs are not involved in ubiquinone biosynthesis in L. erythrorhizon.

The $K_m$ values of LePGT-1 to both substrates are apparently different from those of LePGT-2. As the $K_m$ values of native LePGT were reported to be 18.4 $\mu$m for 4HB and 13.8 $\mu$m for GPP (16), which are similar to those of LePGT-1 (10.3 and 5.1 $\mu$m, respectively), but a direct comparison is not appropriate, because the $K_m$ values of native enzyme were determined with a partially purified protein, and thus it may be a mixture of LePGT-1 and -2. It is not clear whether these two gene products play different roles in vivo, but their expression patterns in L. erythrorhizon are very similar (Figs. 4–6). They might be expressed in different cell types in root tissue.

LePGT polypeptides are predicted to have 8 or 9 transmembrane domains based on a hydropathy analysis by Kyte and Doolittle and a program on CBS servers (www.cbs.dtu.dk/services/TMHMM-1.0/). There are two obvious hydrophilic loops where the ND$\cdot$D motif and GXX$(K/Y)$STAL motif are localized. They are presumed to be the binding sites for the substrate, i.e. the former is for prenyl diphosphate as seen in prenyl diphosphate synthases, and the latter is probably important for the aromatic substrate (39). More detailed analyses are needed to define the differences in the structure that contribute to the strict substrate specificity of LePGT for GPP.

The phylogenetic tree is shown in Fig. 7. Bacterial polyprenyltransferases tend to form a cluster, whereas eukaryotic polyprenyltransferases do not. This is at least partly attributable to the divergence of the mitochondrial sorting signal sequence in each molecular species. In particular, a plant ortholog of LePGT, Arabidopsis polyprenyltransferase, seems to be more divergent than those of unicellular eukaryotes and Caenorhabditis elegans (46). Although the mitochondrial targeting signal is absent in LePGT polypeptides, they may be derived from such prenyltransferase for ubiquinone biosynthesis. It is presumed that during evolution they lost the mitochondrial signal to localize in the ER membrane, which is advantageous both for the biosynthesis and secretion of shikonin out of cells, and gained substrate specificity for GPP. Since most organisms need ubiquinone to survive in the wild, Lithospermum cells should also have another 4HB-prenyltransferase localized in mitochondria. In fact, four to five bands were observed in the genomic Southern blot (data not shown).

Northern analyses revealed that the modes of regulation of gene expression were completely consistent with that of geranyltransferase activity using both positive and negative regulators (Figs. 4–6). The other biosynthetic genes involved in shikonin biosynthesis, such as phenylalanine ammonia-lyase (45) and 4-coumarate CoA ligase (47) which may be also involved in lithospermic acid B production in this plant (48), did not show such a close correlation with shikonin accumulation. This indicates that LePGTs play a pivotal role in the regulation of shikonin biosynthesis at the gene expression level. Due to these attractive properties, LePGTs are suitable for use as model genes to study the molecular mechanism of dark-inducible secondary metabolism, and also its regulation by auxins and elicitors in higher plants.

In contrast to prenyltransferases involved in chain elongation of prenyl diphosphate, those involved in 4HB prenylation have not yet been subjected to molecular analyses. LePGTs isolated in this study are the first cDNAs that encode plant prenyltransferases which catalyze the transfer of the prenyl moiety to an aromatic substrate. They could be very powerful tools for characterizing prenylation of the aromatic moiety at a molecular level, e.g. identification of the 4HB-binding site. An interesting feature of these prenyltransferases is their clear preference for GPP as the substrate; this should be further characterized using chimeric enzymes with prenyltransferases

**Fig. 6.** Organ-specific accumulation of LePGT-1 and LePGT-2 in the intact plant of L. erythrorhizon. LR, lateral root; MR, main root; St, stem; Lf, leaf. The contents of shikonin derivatives recovered from each plant organ are shown at the bottom.

**Fig. 7.** Phylogenetic relationship of 4HB-prenyltransferases including LePGT-1 and -2 reported in the present study. The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) tree was calculated using Gene-Works software. Scores shown on the horizontal lines are the number of mismatches divided by the length of the shorter sequence. Aa, Aquifex aeolicus; Af, Archaeoglobus fulgidus; At, A. thaliana; Bf, Bacillus firmus; Ce, C. elegans; Ct, Chlamydia trachomatis; Ec, E. coli; Hp, Helicobacter pylori; Ps, Providencia stuartii; Rp, Richettsia prowazekii; Sc, S. cerevisiae; Sp, Schizosaccharomyces pombe; Ss, Synechocystis sp.
that show broad substrate specificity, such as 4HPT. From a biotechnological point of view, these cDNAs are expected to be useful for genetic engineering of shikonin production (49), since geranylation is a “bottle-neck” reaction in shikonin biosynthesis. Furthermore, as heterologous probes, these cDNAs may lead to breakthroughs in molecular genetic studies on the secondary metabolism of cannabinoid and prenylated flavonoids and coumarins, which involve prenyltransferase reactions.

Acknowledgments—We are grateful to Dr. W. Frommer, Tubingen University, and Dr. T. Miyakawa, Hiroshima University, for the gift of pDR196 and yeast strain W303-1A, respectively. We thank Dr. H. Tamaki and Dr. Y. Sakai, Graduate School of Kyoto University, for technical suggestions regarding gene disruption in yeast and marker enzyme measurement, respectively. We also thank Dr. K. Hahlbrock and Dr. J. Gershenzon, Max Planck Institute in Cologne and Jena, respectively, for a critical reading of the manuscript. Intact L. erythrohizon plants were a generous gift from the Kyoto Botanical Garden of Takeda Chemical Industries.

REFERENCES

1. Grunler, J., Ericsson, J., and Dalner, G. (1994) Biochim. Biophys. Acta 1212, 259–277
2. Tahara, S., and Ibrahim, R. K. (1995) Phytochemistry 38, 1073–1094
3. Morandi, D. (1996) Plant Soil 185, 241–251
4. Wang, B. H., Ternai, B., and Polya, G. (1997) Phytochemistry 43, 369–375
5. Henderson, M. C., Miranda, C. L., Stevens, J. F., Deinzer, M. L., and Buhler, D. R. (2000) Phytochemistry 53, 1094–1109
6. Yamamoto, H., Inoue, K., and Yazaki, K. (2000) Phytochemistry 54, 649–655
7. Schroeder, G., Zähringer, U., Heller, W., Eitel, J., and Grisebach, H. (1979) Arch. Biochem. Biophys. 194, 635–636
8. Welle, R., and Grisebach, H. (1987) FEBS Lett. 220, 223–226
9. Linsmaier, E. M., and Skoog, F. (1965) Phytochemistry 3, 227–239
10. Laflamme, P., Khouri, H., Gulick, P., and Ibrahim, R. K. (1995) Phytochemistry 34, 147–151
11. Dhillon, D. S., and Brown, S. A. (1976) Arch. Biochem. Biophys. 177, 74–83
12. Hamerski, D., and Matern, U. (1988) Eur. J. Biochem. 171, 569–575
13. Fellermeier, M., and Zenk, M. H. (1998) FEBS Lett. 427, 283–285
14. Mühlenweg, A., Melzer, M., Li, S-M., and Heide, L. (1998) Planta 205, 407–413
15. Tabata, M. (1996) Plant Tissue Culture Lett. 13, 117–125
16. Brigham, L. A., Michaels, P. J., and Flores, H. E. (1999) Plant Physiol. 119, 417–428
17. Yamaoto, H., Sendai, M., and Inoue, K. (2000) Phytochemistry 54, 649–655
18. Schröder, G., Zähringer, U., Heller, W., Ebel, J., and Grisebach, H. (1979) Arch. Biochem. Biophys. 194, 635–636
19. Inouye, H., Ueda, S., Inoue, K., and Matsumura, H. (1979) Phytochemistry 18, 1301–1308
20. Yamaoto, H., Matsuoka, H., Ujihara, T., and Sato, F. (1999) Plant Biotechnol. 16, 335–342
21. Heide, L., Nishioka, N., Fukui, H., and Tabata, M. (1989) Phytochemistry 28, 1873–1877
22. Gaiser, S., and Heide, L. (1996) Phytochemistry 41, 1065–1072
23. Yazaki, K., Matsuoka, H., Shimomura, K., Beechhold, A., and Sato, F. (2001) Plant Physiol. 125, 1831–1841
24. Tani, M., Takeda, K., Yazaki, K., and Tabata, M. (1993) Phytochemistry 34, 1285–1290
25. Yazaki, K., Takeda, K., and Tabata, M. (1997) Plant Cell Physiol. 38, 776–782
26. Ashby, M. N., Kutsunai, S. Y., Ackerman, S., Tragoloff, A., and Edwards, P. A. (1992) J. Biol. Chem. 267, 4128–4136
27. Tabata, M., and Fujita, Y. (1989) in Bio/Technology in Plant Science (Zaitlin, M., Day, P., and Hollaender, A., eds.) pp. 207–218, Academic Press, Orlando, Fl.
28. Welle, R., and Grisebach, H. (1991) Plant Tissue Culture Lett. 10, 343–347
29. Fujita, Y., Haru, Y., Suga, C., and Morimoto, T. (1981) Plant Cell Rep. 1, 61–63
30. Biggs, D. R., Welle, R., Visser, F. R., and Grisebach, H. (1987) Plant Tissue Culture Lett. 13, 117–125
31. Gu, S-M., and Heide, L. (1998) Planta 211, 436–443
32. Kuhlemeier, A., Melzer, M., Li, S-M., and Heide, L. (2000) Nature 407, 611–616
33. Emanuelsson, O., Henrik Nielsen, H., Brunak, S., and von Heijne, G. (2000) J. Mol. Biol. 300, 1005–1016
34. Yamamoto, H., Nakashima, K., Fukui, H., and Tabata, M. (1993) Phytochemistry 32, 633–638
Geranyl Diphosphate:4-Hydroxybenzoate Geranyltransferase from Lithospermum erythrorhizon: CLONING AND CHARACTERIZATION OF A KEY ENZYME IN SHIKONIN BIOSYNTHESIS
Kazufumi Yazaki, Miyuki Kunihisa, Takahiro Fujisaki and Fumihiko Sato

J. Biol. Chem. 2002. 277:6240-6246.
doi: 10.1074/jbc.M106387200 originally published online December 14, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M106387200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 48 references, 6 of which can be accessed free at
http://www.jbc.org/content/277/8/6240.full.html#ref-list-1