Plant Physiol. (1994) 105: 579–583

Isolation and Characterization of cDNAs Encoding Imidazoleglycerolphosphate Dehydratase from *Arabidopsis thaliana*

Sachiyo Tada, Sandra Volrath, David Guyer, Alfred Scheidegger[1], John Ryals, Daisaku Ohta, and Eric Ward*

International Research Laboratories, Ciba (Japan) Ltd., P.O. Box 1, Takarazuka 665 Japan (S.T., A.S., D.O.); and Ciba Agricultural Biotechnology, P.O. Box 12257, Research Triangle Park, North Carolina 27709 (S.V., D.G., J.R., E.W.)

cDNA clones encoding imidazoleglycerolphosphate dehydratase (IGPD; EC 4.2.1.19) from *Arabidopsis thaliana* were isolated by complementation of a bacterial auxotroph. The predicted primary translation product shared significant identity with the corresponding sequences from bacteria and fungi. As in yeast, the plant enzyme is monofunctional, lacking the histidinol phosphatase activity present in the *Escherichia coli* protein. IGPD mRNA was present in major organs at all developmental stages assayed. The *Arabidopsis* genome appears to contain two genes encoding this enzyme, based on DNA gel blot and polymerase chain reaction analysis.

Many metabolic pathways in plants are still rather poorly understood. Amino acid biosynthesis in plants is generally assumed to operate as in bacteria or yeast; direct evidence for the existence of several steps has been amassed in recent years (Brears and Coruzzi, 1991). For practical reasons, the most intensively studied reactions have been those blocked by inhibitor compounds that are able to act as herbicides in vivo. Examples of enzymes known to be herbicide targets are enolpyruvylshikimate phosphate synthase, acetohydroxyacid synthase, and Gln synthetase (Kishore and Shah, 1988).

His biosynthesis has only recently been studied in detail in plants. The cDNA for histidinol dehydrogenase, which catalyzes the last two steps in the pathway, was isolated based on peptide sequences from the purified enzyme (Nagai et al., 1991), providing the first direct evidence that the biosynthetic pathway in plants is like that in microbes. Here we describe the isolation of the first plant cDNAs encoding IGPD, which converts IGP to IAP en route to His biosynthesis.

MATERIALS AND METHODS

Escherichia coli strain SB3930 (*hisB463*, λ^-; CGSC No. 4930) (Garrick-Silversmith and Hartman, 1970) was obtained from the *E. coli* Genetics Stock Center and was rendered F^+ by mating it with *E. coli* strain K603 (*thr-1, leuB6, trpE63, thi-1, ara-14, lacY1, galK2, galT22, xyl-5, mtl-1, supE44*, λ^-; CGSC No. 6451), which harbors F1::Tn10, conferring tetracycline resistance. A tetracycline resistant his^- exconjugant was selected and named ST1.

A cDNA library of leaf tissue of *Arabidopsis thaliana* ecotype Columbia (Uknes et al., 1992) constructed in the vector λZAPII (Stratagene) was converted to pBluescript phagemid particles by superinfection with f1 helper phage strain R408. A 90-μL aliquot of the cDNA library phagemid stock (2.1×10^6 transducing units/mL) was mixed with 2.2 mL of a mid-log phase culture of strain ST1. The mixture was incubated at 37°C for 15 min, then pelleted and washed in minimal E medium (Vogel and Bonner, 1956). The washed cells were plated on E plates (containing ampicillin at 100 μg/mL) and incubated for 2 d at 37°C. his^+ colonies were purified by restreaking, and their plasmids were extracted and tested for ability to transform ST1 to his^+ at high frequency.

Extracts of soluble protein from *E. coli* strains ST1(pBluescript), ST1(pSTA3), and ST1(pSTA4), and XL1-Blue (Stratagene), a his^+ strain, were prepared as follows. The bacteria were grown overnight in LB medium or, for XL1-Blue, in E medium, and collected by centrifugation. Approximately 0.5 to 1.0 g of cells was resuspended in 4 mL of 100 mM triethanolamine (pH 7.5), 100 mM 2-mercaptoethanol, 1 mM $MnCl_2$, and broken by sonication in short pulses for 2 to 3 min. Cell debris was removed by centrifugation and proteins in the supernatant were precipitated by addition of $(NH_4)_2SO_4$ to a concentration of 80% (w/v). The protein pellet was redissolved in the buffer described above and desalted by passage over a Sephadex G-25 column (Pharmacia). Extracts were assayed for IGPD activity as described (Mano et al., 1993) by measuring imidazoleacetol obtained after enzymic hydrolysis of IAP.

Plasmids containing cDNA inserts were purified using Magic Miniprep columns (Promega). DNA sequencing was carried out on double-stranded templates using the chain-termination method with fluorescent-labeled dideoxynucleoside triphosphates (Applied Biosystems). RNA and DNA gel-blot experiments were carried out as described (Ausubel et al., 1987). Alignment of IGPD protein sequences was carried out using program PILEUP (Genetics Computer Group).

The genomic sequence corresponding to pSTA3 was am-

[1] Present address: Swiss Center for Scientific Computing, Via Cantonale, CH-6928 Manno, Switzerland.

* Corresponding author; fax 1-919-541-8557.

Abbreviations: IAP, imidazoleacetolphosphate; IGP, imidazoleglycerolphosphate; IGPD, imidazoleglycerolphosphate dehydratase; PIR, Protein Information Resource.

plified using Amplitaq (Perkin-Elmer, Norwalk, CT), genomic DNA from *A. thaliana* ecotype Columbia, and oligonucleotide primers designed from each end of the cDNA sequence (forward primer, beginning at nucleotide 2: 5′-TTCCTTCCGCTGCCAAC; reverse primer, beginning at nucleotide 1129: 5′-AAGCAAATTACAGTCAC).

RESULTS

We attempted to isolate a cDNA for plant IGPD by functionally complementing a bacterial His auxotroph that lacked this enzyme activity. IGPD activity in *E. coli* occurs as part of a bifunctional polypeptide that also has histidinol phosphatase activity. Thus, most *hisB* mutants lack both activities and would not be complemented should the plant gene encode a monofunctional protein. This was considered likely, because the purified enzyme from wheat germ possesses no phosphatase activity (Mano et al., 1993). The *hisB*463 allele specifically eliminates dehydratase activity; strain SB3930 containing this mutation had previously been used to clone the yeast IGPD-encoding *HIS3* gene by complementation (Struhl et al., 1976; Struhl and Davis, 1977). Moreover, the *hisB*463 allele was not known to revert to wild type.

A phagemid library comprising *Arabidopsis* leaf cDNAs was infected into strain ST1 (an F^+ derivative of SB3930) and the infected culture was plated on minimal agar plates. Two prototrophic colonies were isolated from a culture infected with approximately 10^5 transducing units from the library. The individual colonies were purified by restreaking them on minimal medium and their plasmids were tested for their ability to transform ST1 to His prototrophy at high frequency. Plasmid isolates from both prototrophic colonies transformed ST1 to his^+ and ampicillin resistance at approximately equal frequencies, indicating that the basis for His-independent growth was plasmid borne. The inserts of the two plasmids (designated pSTA3 and pSTA4) cross-hybridized and were approximately 1.1 kb in length (data not shown).

To confirm that the cDNA encoded a functional IGPD enzyme, extracts of *E. coli* ST1 harboring pSTA3 and pSTA4, ST1 harboring the pBluescript vector, and *E. coli* XL1-Blue (his^+) were assayed for IGPD activity (Table I). Extracts from cultures of XL1-Blue, ST1(pBluescript), ST1(pSTA3), and ST1(pSTA4) were prepared and their IGPD activity was assayed in vitro. ST1(pBluescript) contained no detectable activity, but ST1(pSTA3) and ST1(pSTA4) extracts contained levels of activity comparable to that seen in XL1-Blue.

The sequences of the cDNA inserts of pSTA3 and pSTA4 were determined and found to be identical except for the length of the poly(A) tail and the presence of a duplicated *Eco*RI linker at the 5′ end of the pSTA4 insert. Thus, the two

Table I. *IGPD activity in strains of E. coli*

Strain	IGPD Activity
	nmol mg^{-1} *protein* h^{-1}
XL1-Blue	122
ST1(pBluescript)	0
ST1(pSTA3)	99
ST1(pSTA4)	138

```
1    GTTCCTTCCGCTGCCAACAAAATGGAGCTGTCGTCTGCGTCCGCCATATTAAGCCACTCC   60
                     M  E  L  S  S  A  S  A  I  L  S  H  S
61   TCCTCCGCCGCTCAGCTTCTCAGACCTAAGCTCGGGTTTATTGATTTGCTTCCTCGTCGA  120
     S  S  A  A  Q  L  L  R  P  K  L  G  F  I  D  L  L  P  R  R
121  GCGATGATCGTTTCTTCTCCTTCTTCTTCGCTTCCTCGATTTTTGCGGATGGAATCTCAA  180
     A  M  I  V  S  S  P  S  S  S  L  P  R  F  L  R  M  E  S  Q
181  TCTCAGCTTCGCCAATCTATCTCTTGCTCTGCTTCTTCTTCTTCTTCTATGGCATTAGGT  240
     S  Q  L  R  Q  S  I  S  C  S  A  S  S  S  S  S  M  A  L  G
241  AGAATTGGAGAAGTAAAGAGAGTAACAAAGGAAACGAATGTTTCAGTGAAGATTAATTTG  300
     R  I  G  E  V  K  R  V  T  K  E  T  N  V  S  V  K  I  N  L
301  GATGGTACTGGAGTTGCAGATAGTTCTAGTGGAATTCCTTTCCTTGACCATATGTTAGAT  360
     D  G  T  G  V  A  D  S  S  S  G  I  P  F  L  D  H  M  L  D
361  CAACTTGCTTCGCATGGCTTGTTTGATGTGCACGTTAGAGCTACTGGTGATGTTCACATT  420
     Q  L  A  S  H  G  L  F  D  V  H  V  R  A  T  G  D  V  H  I
421  GATGATCATCACACTAATGAAGATATAGCTCTTGCCATTGGAACTGCTCTATTAAAGGCT  480
     D  D  H  H  T  N  E  D  I  A  L  A  I  G  T  A  L  L  K  A
481  CTTGGTGAGCGTAAAGGGATTAACCGGTTTGGTGACTTCACAGCTCCTCTAGATGAAGCG  540
     L  G  E  R  K  G  I  N  R  F  G  D  F  T  A  P  L  D  E  A
541  CTTATACATGTTTCCTTGGACTTGTCTGGTCGACCATATCTTGGTTACAACTTGGAGATA  600
     L  I  H  V  S  L  D  L  S  G  R  P  Y  L  G  Y  N  L  E  I
601  CCAACTCAGAGAGTTGGAACATATGATACTCAGTTGGTGGAGCACTTTTTCCAGTCGTTG  660
     P  T  Q  R  V  G  T  Y  D  T  Q  L  V  E  H  F  F  Q  S  L
661  GTGAATACTTCTGGTATGACTCTTCACATTCGGCAGCTCGCTGGTGAAAACTCTCATCAC  720
     V  N  T  S  G  M  T  L  H  I  R  Q  L  A  G  E  N  S  H  H
721  ATAATAGAGGCGACGTTTAAGGCGTTTGCCAGAGCTCTACGACAAGCAACAGAGACTGAT  780
     I  I  E  A  T  F  K  A  F  A  R  A  L  R  Q  A  T  E  T  D
781  CCACGCCGTGGTGGGACAATACCAAGTTCAAAAGGAGTCTTATCACGGTCTTGAAAGCTA  840
     P  R  R  G  G  T  I  P  S  S  K  G  V  L  S  R  S  *
841  ATCAAACACACAAGACAGTTCCCAGATTCACACTTCATCGTCGAGTTCATGAGCCATCGT  900
901  CAATTCTCTTATGGTACCAAATGCCAAGCCTGTTGGATCTTGCTGTTCCATTCCATTACA  960
961  GAAGCACAAAGAGCAAAATGTGAAAATAGATTAGAGATCACACAGTTCAGAAGATCATAG  1020
1021 GCTCATCTTTATATTAATCTGTTGTTGCAGAGTGTATTAAACCTCTTACCATTGCTGTAT  1080
1081 CATCATCAACTGAGAACTTACTGTGAGTTGAAGTGACTGTAATTTGCTTTAAAAAAAAAA  1140
1141 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  1174
```

Figure 1. The *A. thaliana* IGPD cDNA sequence.

cDNAs represent independent cloning events. The sequence of the pSTA3 insert appears in Figure 1. When compared to the *hisB* gene of *E. coli* and the *HIS3* gene of *Saccharomyces cerevisiae* by dot matrix analysis, the *Arabidopsis* cDNA showed significant homology to both sequences (data not shown). The cDNA contains an open reading frame of 270 codons, predicted to encode a protein with 41 and 44% identity to the *E. coli* and yeast enzymes, respectively.

Comparison of IGPD protein sequences from a variety of organisms revealed a high degree of similarity (Fig. 2). A partial cDNA isolated from wheat (S. Volrath, D. Guyer, and E. Ward, unpublished data; GenBank accession No. U02690) was 87% identical in predicted protein sequence to the *Arabidopsis* sequence. Remarkably, the predicted protein sequence encoded by an *Anabaena* gene assumed to be *hisB* (Wei et al., 1993) was 67% identical to *Arabidopsis*. The precise N terminus of mature plant IGPD has not yet been determined; the enzyme from wheat germ is blocked to N-terminal Edman degradation (Mano et al., 1993). However, the predicted *Arabidopsis* translation product contains an apparently conserved Arg residue at position 74, suggesting that the N terminus of the mature protein occurs at or before this residue.

As judged by RNA gel-blot analysis, the *Arabidopsis* mRNA for IGPD is approximately 1.1 kb in length, consistent with the size of the cDNA clone isolated (data not shown). To assess IGPD expression, mRNA levels in various organs harvested throughout development (Potter et al., 1993) were analyzed by gel-blot hybridization. Nearly identical levels of IGPD mRNA were detected in all samples (Fig. 3).

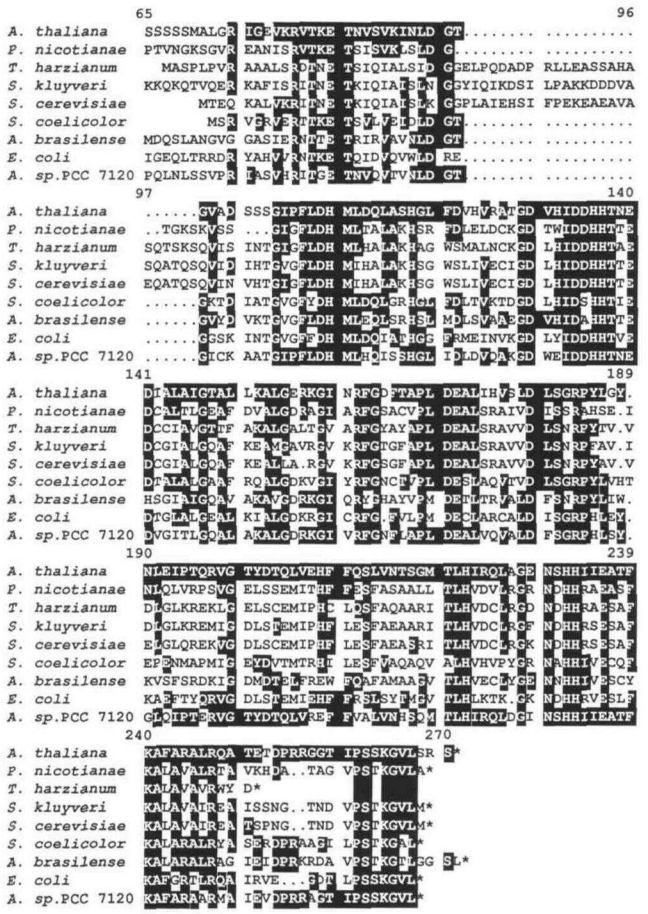

Figure 2. Alignment of predicted protein sequences from known IGPD genes. Amino acid residues identical to the plant sequence are shaded. Periods indicate regions where gaps were inserted to improve alignment. The sequences are from: *A. thaliana*, beginning with codon 65 of the open reading frame shown in Figure 1; *Phytophthora nicotianae*, beginning with residue 251 (PIR accession No. S22199); *Trichoderma harzianum*, from residue 1 (Goldman et al., 1992) (PIR accession No. S26196); *Saccharomyces kluyveri*, from residue 7 (Weinstock and Strathern, 1993) (PIR accession No. S31235); *Saccharomyces cerevisiae*, from residue 1 (Struhl, 1985) (Swiss-Prot accession No. P06633); *Streptomyces coelicolor*, from residue 1 (Limauro et al., 1990) (Swiss-Prot accession No. P16247); *Azospirillum brasilense*, from residue 1 (Fani et al., 1989) (Swiss-Prot accession No. P18787); *E. coli*, from residue 158 (Chiarotti et al., 1986b) (Swiss-Prot accession No. P06987); *Anabaena* sp. strain PCC 7120, from residue 7 (Wei et al., 1993) (ORF2 of GenBank accession No. L10036).

To examine the number of IGPD genes in *Arabidopsis*, a gel blot of genomic DNA digested with several enzymes was probed with the cDNA insert of pSTA3. With the use of enzymes that do not cut within the cDNA (*Bam*HI, *Bgl*II, *Hin*dIII), two bands were reproducibly detected in genomic DNA, indicating that possibly two IGPD genes may be present in the *Arabidopsis* genome (Fig. 4). Three additional enzymes generated more than two fragments that were detected with the IGPD probe. *Dra*I digestion gave rise to three bands. This enzyme cuts once at the very end of the cDNA,

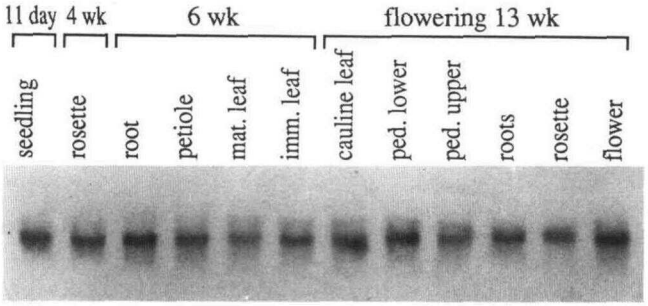

Figure 3. Expression of IGPD mRNA throughout *A. thaliana* development. A gel blot of total RNA isolated at the times after planting indicated was probed with the IGPD cDNA shown in Figure 1. Four- and 6-week-old plants had not initiated flowering under the SD growth conditions used. mat. leaf, Mature, fully expanded leaves without petioles; imm. leaf, immature, smaller rosette leaves; ped. lower, peduncles, lower portion; ped. upper, peduncles, upper portion including pedicels.

in a sequence partially composed of poly(A); thus, this site is not likely to occur in genomic DNA. *Eco*RI, which cuts the cDNA once, also gave rise to three bands on the genomic Southern blot. *Xba*I, which also cuts the cDNA once, gave rise to four hybridizing fragments.

To further characterize the origin of the fragments detected in the genomic blot, and to determine more accurately the number of IGPD genes, PCR amplification was performed on genomic DNA using oligonucleotide primers designed from the 5′ and 3′ untranslated sequences of the cDNA. A single product approximately 2.1 kb in length was detected, indicating that the gene corresponding to the cDNA contained at least one intron. Results of digestion of the fragment with each of the enzymes used in the Southern blot experiment are summarized in Table II. *Bgl*II and *Hin*dIII did not cut the fragment, consistent with the presence of two IGPD genes in the *Arabidopsis* genome. *Dra*I or *Eco*RI digestion of the PCR product generated two fragments, and *Xba*I digestion

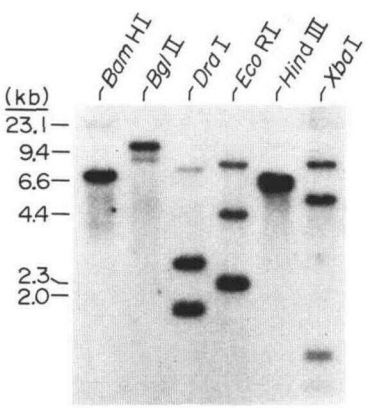

Figure 4. Southern blot of *A. thaliana* Columbia genomic DNA digested with the restriction endonucleases indicated and probed with the cDNA shown in Figure 1. The positions of size standards are indicated. The *Hin*dIII band is a doublet on less dense autoradiograms.

Table II. *Fragments generated from restriction digestion of a 2.1-kb fragment of genomic DNA amplified using primers from each end of the IGPD cDNA*

Enzyme	Fragment Size
	kb
*Bam*HI	ND[a]
*Bgl*II	2.1
*Dra*I	1.6, 0.5
*Eco*RI	1.7, 0.4
*Hind*III	2.1
*Xba*I	1.1, 0.7, 0.3

[a] ND, Not determined.

gave three fragments. These results indicated that the intron(s) in the gene represented by the cDNA contains a single site for both *Dra*I and *Xba*I. Thus, the number of bands detected in the genomic Southern analysis is consistent with the assumption of two IGPD genes in *Arabidopsis*.

DISCUSSION

Our results, combined with the previous isolation of the cDNA for histidinol dehydrogenase, illustrate that the biosynthesis of His proceeds in plants as it does in bacteria and fungi. The organization of the genes for His biosynthesis in plants shares aspects of both the prokaryotic and yeast systems. In plants and yeast, IGPD activity resides on a single polypeptide, but bacterial IGPD occurs as a bifunctional protein in combination with histidinol phosphatase (Struhl, 1985; Chiarotti, 1986b). In contrast, yeast histidinol dehydrogenase is part of a trifunctional protein (also containing phosphoribosyl-AMP cyclohydrolase and phosphoribosyl-ATP pyrophosphohydrolase) (Donahue et al., 1982), whereas plants and prokaryotes maintain histidinol dehydrogenase as a single protein lacking other His biosynthetic activities (Chiarotti et al., 1986a; Nagai et al., 1991).

Structural features of the cDNA from *A. thaliana* are consistent with localization of the protein in chloroplasts. The open reading frame extended approximately 73 codons 5′ to the region in which extensive homology is seen, compared to IGPD sequences from other organisms. This putative N-terminal extension has features characteristic of chloroplast transit peptides (von Heijne and Nishikawa, 1991); specifically, it is rich in Ser (22/73) and has few negatively charged residues (3/73). Presumably, the entire His biosynthetic pathway is plastid localized, because histidinol dehydrogenase is also believed to be targeted to the chloroplast (Nagai et al., 1991).

The mechanism by which IGPD catalyzes the dehydration reaction is unknown. Other proteins with similar activities contain iron-sulfur centers that are presumed to be involved in catalysis (Walsh, 1979). The lack of conserved Cys residues in IGPD indicates that a sulfur-ligated iron is not present in the protein and that the enzyme-catalyzed dehydration of IGP may occur by a mechanism distinct from other enzymic dehydrations, such as the reactions catalyzed by aconitase (Lauble et al., 1992) and dihydroxyacid dehydratase (Flint et al., 1993). In addition, the unusually high concentration of 2-mercaptoethanol required for maximal activity of wheat IGPD must be necessary for some function other than sulfhydryl group protection (Mano et al., 1993).

If two IGPD genes are in fact present and expressed in *Arabidopsis*, their relevance to regulation of gene expression and metabolite flow is not clear. Gene pairs encoding enzymes for two steps in Trp biosynthesis are subject to differential induction by pathogen infection (Keith et al., 1991; Niyogi and Fink, 1992). We found no evidence for developmental or organ-specific expression in *Arabidopsis* using the full-length IGPD cDNA probe. However, gene-specific expression analysis may reveal differential regulation in response to various physiological, developmental, or environmental stimuli.

ACKNOWLEDGMENTS

We thank V. Jo Davisson for valuable discussions, Sharon Potter for RNAs isolated from various organs and developmental stages, the *E. coli* Genetic Stock Center for strains SB3930 and K603, and Tim Brears, Bruce Lee, Hans-Peter Schär, Murray Spruill, and Scott Uknes for critically reading the manuscript.

Received October 29, 1993; accepted February 20, 1994.
Copyright Clearance Center: 0032-0889/94/105/0579/05.
The GenBank accession number for the sequence reported in this article is U02689.

LITERATURE CITED

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1987) Current Protocols in Molecular Biology. John Wiley & Sons, New York

Brears T, Coruzzi GM (1991) The molecular biology of amino acid biosynthesis in plants. *In* JK Setlow, ed, Genetic Engineering, Vol 13. Plenum Press, New York, pp 221–236

Chiarotti L, Alifana P, Carlomagno MS, Bruni CB (1986a) Nucelotide sequence of the *Escherichia coli hisD* gene and of the *Escherichia coli* and *Salmonella typhimurium hisIE* region. Mol Gen Genet **203:** 382–388

Chiarotti L, Nappo AG, Carlomagno MS, Bruni CB (1986b) Gene structure in the histidine operon of *Escherichia coli*: identification and nucleotide sequence of the *hisB* gene. Mol Gen Genet **202:** 42–47

Donahue TF, Farabaugh PJ, Fink GR (1982) The nucleotide sequence of the *HIS4* region of yeast. Gene **18:** 47–59

Fani R, Bazzicalupo M, Damiani G, Bianchi A, Schipani C, Sgaramella V, Polsinelli M (1989) Cloning of histidine genes of *Azospirillum brasilense*: organization of the *ABFH* gene cluster and nucleotide sequence of the *hisB* gene. Mol Gen Genet **216:** 224–229

Flint D, Emptage MH, Finnegan MG, Fu W, Johnson MK (1993) The role and properties of the iron-sulfur cluster in *Escherichia coli* dihydroxy-acid dehydratase. J Biol Chem **268:** 14732–14742

Garrick-Silversmith L, Hartman PE (1970) Histidine-requiring mutants of *Escherichia coli* K12. Genetics **66:** 231–244

Goldman GH, Demolder J, Dewaele S, Herrera-Estrella A, Geremia RA, Van Montagu M, Contreras R (1992) Molecular cloning of the imidazolegylcerolphosphate dehydratase gene of *Trichoderma harzianum* by genetic complementation in *Saccharomyces cerevisiae* using a direct expression vector. Mol Gen Genet **234:** 481–488

Keith B, Dong X, Ausubel FM, Fink GR (1991) Differential induction of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase genes in *Arabidopsis thaliana* by wounding and pathogenic attack. Proc Natl Acad Sci USA **88:** 8821–8825

Kishore GM, Shah DM (1988) Amino acid biosynthesis inhibitors as herbicides. Annu Rev Biochem **57:** 627–663

Lauble H, Kennedy MC, Beinert H, Stout CD (1992) Crystal structures of aconitase with isocitrate and nitroisocitrate bound. Biochemistry **31:** 2735–2748

Limauro D, Avitabile A, Cappellano M, Puglia AM, Bruni CB (1990) Cloning and characterization of the histidine biosynthetic gene cluster of *Streptomyces coelicolor* A3(2). Gene **90:** 31–41

Mano J, Hatano M, Koizumi S, Tada S, Hashimoto M, Scheidegger A (1993) Purification and properties of a monofunctional imidazoleglycerol-phosphate-dehyratase from wheat. Plant Physiol **103:** 733–739

Nagai A, Ward E, Beck J, Tada S, Chang J-Y, Scheidegger A, Ryals J (1991) Structural and functional conservation of histidinol dehydrogenase between plants and microbes. Proc Natl Acad Sci USA **88:** 4133–4137

Niyogi KK, Fink GR (1992) Two anthranilate synthase genes in *Arabidopsis*: defense-related regulation of the tryptophan pathway. Plant Cell **4:** 721–733

Potter S, Uknes S, Lawton K, Winter AM, Chandler D, DiMaio J, Novitzky R, Ward E, Ryals J (1993) Regulation of a hevein-like gene in *Arabidopsis*. Mol Plant Microbe Interact **6:** 680–685

Struhl K (1985) Nucleotide sequence and transcriptional mapping of the yeast *pet56-his3-ded1* gene region. Nucleic Acids Res **13:** 8587–8601

Struhl K, Cameron JR, Davis RW (1976) Functional genetic expression of eukaryotic DNA in *Escherichia coli*. Proc Natl Acad Sci USA **73:** 1471–1475

Struhl K, Davis RW (1977) Production of a functional eukaryotic enzyme in *Escherichia coli*: cloning and expression of the yeast structural gene for imidazoleglycerolphosphate dehydratase (*his3*). Proc Natl Acad Sci USA **74:** 5255–5259

Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D, Slusarenko A, Ward E, Ryals J (1992) Acquired resistance in *Arabidopsis*. Plant Cell **4:** 645–656

Vogel HJ, Bonner DM (1956) Acetylornithase of *Escherichia coli*: partial purification and some properties. J Biol Chem **218:** 97–106

von Heijne G, Nishikawa K (1991) Chloroplast transit peptides: the perfect random coil? FEBS Lett **278:** 1–3

Walsh C (1979) Enzymatic Reaction Mechanisms. WH Freeman, New York

Wei TF, Ramasubramanian TS, Pu F, Golden JW (1993) *Anabaena* sp. strain PCC 7120 *bifA* gene encoding a sequence-specific DNA binding protein cloned by in vivo transcriptional interference selection. J Bacteriol **175:** 4025–4035

Weinstock KG, Strathern JN (1993) Molecular genetics in *Saccharomyces kluyveri*: the *HIS3* homolog and its use as a selectable marker gene in *S. kluyveri* and *Saccharomyces cerevisiae*. Yeast **9:** 351–361