Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4133–4137, May 1991
Genetics

Structural and functional conservation of histidinol dehydrogenase between plants and microbes

(amino acid biosynthesis/*Brassica oleracea*/cDNA cloning/polymerase chain reaction)

ATSUKO NAGAI*, ERIC WARD†, JAMES BECK†, SACHIYO TADA*, JUI-YOA CHANG‡, ALFRED SCHEIDEGGER*, AND JOHN RYALS†§

*International Research Laboratories, CIBA-GEIGY (Japan) Ltd., P.O. Box 1, Takarazuka, 665 Japan; †Agricultural Biotechnology Research Unit, CIBA-GEIGY Corporation, P.O. Box 12257, Research Triangle Park, NC 27709; ‡CIBA-GEIGY AG, CH-4002 Basel, Switzerland

Communicated by Mary-Dell Chilton, February 13, 1991 (received for review December 6, 1990)

ABSTRACT **The partial amino acid sequence of histidinol dehydrogenase (L-histidinol:NAD+ oxidoreductase, EC 1.1.1.23) from cabbage was determined from peptide fragments of the purified protein. The relative positions of these peptides were deduced by aligning their sequences with the sequence of the *HIS4C* gene product of *Saccharomyces cerevisiae*. cDNA encoding histidinol dehydrogenase was then amplified from a library using a polymerase chain reaction primed with degenerate oligonucleotide pools of known position and orientation. By using this amplified fragment as a probe, an apparently full-length cDNA clone was isolated that is predicted to encode a proenzyme having a putative 31-amino acid chloroplast transit peptide and a mature molecular mass of 47.5 kDa. The predicted protein sequence was 51% identical to the yeast enzyme and 49% identical to the *Escherichia coli* enzyme. Expression of the cDNA clone in an *E. coli his* operon deletion strain rendered the mutant able to grow in the presence of histidinol.**

In plants, the biosynthetic pathways of most of the amino acids are poorly understood. Only a handful of enzymes involved in amino acid biosynthesis have been purified from plant sources, partly because of the small amount of these proteins found in plant cells. To date, glutamate synthase, glutamate dehydrogenase, and glutamine synthetase in the glutamate pathway (1–3), aspartate kinase (4) and homoserine dehydrogenase (5) in the threonine pathway, dihydrodipicolinate synthase (6) in the lysine pathway, and 3-deoxy-D-arabinoheptulosonate 7-phosphate synthase (7) and 5-enoylpyruvyl shikimate-3-phosphate synthase (8) in the aromatic amino acid pathway have been characterized and purified from various plant species. cDNAs encoding several of these enzymes have been cloned (8–12), as have genes for other amino acid biosynthetic enzymes based on their structural or functional homology to microbial or mammalian enzymes (13–17).

Although histidine biosynthesis has been elucidated in several prokaryotic and eukaryotic microbes (18–22), the biosynthetic pathway in higher plants is unknown (23). Indirect evidence has indicated that the pathway follows a route similar to that found in bacteria and fungi (24, 25). Recently, histidinol dehydrogenase (HDH; L-histidinol:NAD+ oxidoreductase, EC 1.1.1.23) was purified to homogeneity from *Brassica oleracea* (cabbage; ref. 26), proving that the final steps in histidine biosynthesis proceed in plants as they do in prokaryotes and fungi.

In this report, we describe the isolation of a full-length cDNA encoding HDH from cabbage. The plant coding sequence¶ was approximately 50% identical to known microbial genes. To demonstrate function of the plant clone, the cDNA was expressed in an *Escherichia coli* strain lacking the histidine operon. The cDNA was found to suppress the *his* deletion when the bacteria were grown in the presence of histidinol.

MATERIALS AND METHODS

Plant Material and Bacterial Strains. Mature spring cabbage (*Brassica oleracea* L. var *capitata* L.) was purchased from a local grocer in Japan. Phage vector λ ZAP II and *E. coli* VCS257 were obtained from Stratagene. *E. coli* WB353 (Δ[*his-gnd*]) is a derivative of TA2043 (27) that has been cured of λ phage (28).

Peptide Preparation and Sequencing. HDH was purified from cabbage as described (26). The purified HDH was subjected directly to automated Edman degradation with an Applied Biosystems model 470A gas-liquid-phase protein sequencer (29) to derive amino-terminal sequence from the mature enzyme. The phenylthiohydantoin amino acid derivatives were separated and identified with an on-line phenylthiohydantoin analyzer (Applied Biosystems) fitted with a phenylthiohydantoin C_{18} column.

Peptides generated by lysyl endopeptidase (EC 3.4.21.50; Wako Biochemicals, Osaka) digestion or CNBr cleavage were separated by reverse-phase HPLC on an Aquapore RP-300 (C_8) column (2.1 × 220 mm). The column was eluted with a linear gradient of 0–70% (vol/vol) acetonitrile in 0.1% trifluoroacetic acid. Peptides were collected and analyzed by automated Edman degradation as described above. Further details of the peptide preparation and protein sequencing are available from the authors on request.

Nucleic Acid Preparations. Total RNA was prepared by phenol extraction essentially as described (30). Poly(A)$^+$ mRNA was isolated from total RNA using a Poly(A) Quik mRNA isolation kit (Stratagene). A cDNA library was constructed in the λ ZAP II vector using a ZAP cDNA Gigapack II Gold synthesis kit (Stratagene) according to instructions supplied by the manufacturer. cDNA inserts were excised *in vivo* as subclones in the pBluescript plasmid (Stratagene) and sequenced as double-stranded templates using the dideoxynucleotide chain-termination method (31). Sequences were aligned using the GAP program (32). Phage DNA was prepared with the LambdaSorb reagent (Promega) using instructions supplied by the manufacturer. Plant genomic DNA was prepared as described (33).

Polymerase Chain Reaction. Phage DNA (10 μg) prepared from the cabbage cDNA library was used as template in a reaction mixture of 50 μl. The concentrations of the remaining components were degenerate primers (each at 4 μM), 100

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: HDH, histidinol dehydrogenase.

§To whom reprint requests should be addressed.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M60466).

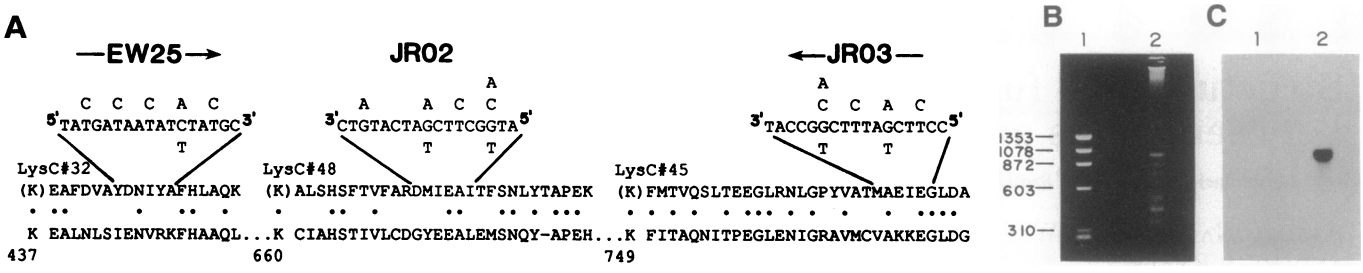

FIG. 1. Amplification of the HDH cDNA. (*A*) Alignment of peptides from the cabbage enzyme (upper line) with portions of the translated *HIS4* coding sequence from yeast (lower line). Positions of identity are marked with dots. Amino acid numbers are given below the yeast sequence. Oligonucleotide primers are shown above the amino acid sequences from which they were derived. (*B*) Ethidium bromide-stained agarose gel showing the products of a polymerase chain reaction primed with EW25 and JR03 (lane 2). The template was total DNA extracted from a cabbage cDNA library. The positions of molecular size markers (lane 1) are indicated in base pairs. (*C*) Autoradiogram of a filter blot of the gel, shown in *B*, probed with the internal primer JR02.

μM dATP, 100 μM dCTP, 100 μM dGTP, 100 μM dTTP, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris·HCl (pH 8.3), and *Amplitaq* DNA polymerase 50 units/ml (Perkin–Elmer/Cetus). By using a Perkin–Elmer/Cetus thermal cycler, the reaction was incubated through 40 cycles of 45 sec at 94°C, 45 sec at 42°C, and 45 sec at 72°C, with 2 sec added to the 72°C incubation period at each cycle.

Blots and Filter Hybridizations. Plaque filter hybridizations were performed as described (34). DNA was separated electrophoretically on agarose gels and blotted to a nylon membrane (GeneScreen*Plus*; NEN) in alkali (35). Hybridization and washing were performed as described (36, 37). RNA gel blot hybridization was essentially as described by Payne *et al.* (34).

```
1    CACGAGCCTATTCGCCGGCGAACACACTCCTATTTGAGCAAACCCGGTGAGGATGTCATTCGATCTATCTCGTCTCTCCCTCACTTCCTCGCCTCGTCTC   100
                                                    M  S  F  D  L  S  R  L  S  L  T  S  S  P  R  L

101  TCGTTTCTCACTCGCACTGCTACTAAGAAAGGATTCGTTAGGTGTTCGATGAAGTCGTACAGATTATCTGAACTTAGTTTCTCTCAAGTTGAGAACTTGA   200
     S  F  L  T  R  T  A  T  K  K  G  F  V  R  C  S  M  K  S  Y  R  L  S  E  L  S  F  S  Q  V  E  N  L  K
                                    N-terminal(A/K)----(I/S)----------(?)----------
                                                        LysC#17(K  T)----------(A/S K)

201  AGGCACGCCCTCGCATTGACTTCTCTTCCATTTTCACCACTGTTAACCCAATCATCGACGCTGTTCGTAGCAAAGGAGATACTGCTGTCAAAGAGTATAC   300
       A  R  P  R  I  D  F  S  S  I  F  T  T  V  N  P  I  I  D  A  V  R  S  K  G  D  T  A  V  K  E  Y  T
     -------(?  ?)-----

301  AGAGAGATTTGACAAAGTCCAGCTCAATAAAGTGGTGGAGGATGTGTCCGAACTTGATATCCCTGAGCTCGACTCCGCAGTTAAAGAAGCGTTTGATGTT   400
       E  R  F  D  K  V  Q  L  N  K  V  V  E  D  V  S  E  L  D  I  P  E  L  D  S  A  V  K  E  A  F  D  V
                                                                    LysC#32----------

401  GCGTATGACAACATTTATGCATTTCACTTTGCCCAAATGTCAACTGAGAAAAGCGTTGAGAATATGAAAGGTGTAAGATGTAAAAGGGTGTCGAGATCTA   500
     A  Y  D  N  I  Y  A  F  H  F  A  Q  M  S  T  E  K  S  V  E  N  M  K  G  V  R  C  K  R  V  S  R  S  I
     -------------------------(L)------(K)

501  TTGGTTCTGTTGGTCTTTATGTGCCTGGTGGAACTGCTGTTTTGCCATCTACTGCTTTGATGCTTGCTATTCCTGCTCAGATTGCTGGATGTAAAACAGT   600
       G  S  V  G  L  Y  V  P  G  G  T  A  V  L  P  S  T  A  L  M  L  A  I  P  A  Q  I  A  G  C  K  T  V
                                                  CNBr#18-------------------------(R)---------
                                                                                LysC#18--------

601  TGTCCTCGCAACTCCACCAACTAAGGAAGGAAGCATATGTAAGGAGGTGCTGTATTGTGCCAAGAGGGCTGGTGTAACTCACATTCTTAAAGCTGGTGGA   700
       V  L  A  T  P  P  T  K  E  G  S  I  C  K  E  V  L  Y  C  A  K  R  A  G  V  T  H  I  L  K  A  G  G
     -------------
     ----------------------

701  GCACAGGCTATAGCTGCCATGGCCTGGGGGACAGATTCTTGTCCTAAGGTTGAGAAGATTTTTGGTCCTGGGAATCAGTATGTTACAGCTGCTAAGATGA   800
       A  Q  A  I  A  A  M  A  W  G  T  D  S  C  P  K  V  E  K  I  F  G  P  G  N  Q  Y  V  T  A  A  K  M  I

801  TTCTGCAAAACAGCGAGGCAATGGTTTCGATTGATATGCCTGCTGGCCCTTCAGAAGTTCTTGTTATCGCTGATGAACATGCTAGTCCAGTTTACATTGC   900
       L  Q  N  S  E  A  M  V  S  I  D  M  P  A  G  P  S  E  V  L  V  I  A  D  E  H  A  S  P  V  Y  I  A

901  AGCCGACTTACTTTCTCAGGCTGAGCATGGTCCAGATAGTCAGGTTGTTCTTGTTGTTGTAGGCGATGGTGTAAATCTCAAAGCCATCGAAGAAGAAATT  1000
       A  D  L  L  S  Q  A  E  H  G  P  D  S  Q  V  V  L  V  V  V  G  D  G  V  N  L  K  A  I  E  E  E  I

1001 GCTAAACAATGCAAAAGCCTTCCTAGAGGCGAGTTTGCTTCAAAAGCTCTAAGTCACAGCTTCACAGTATTTGCTCGAGATATGATTGAGGCAATAACTT  1100
       A  K  Q  C  K  S  L  P  R  G  E  F  A  S  K  A  L  S  H  S  F  T  V  F  A  R  D  M  I  E  A  I  T  F
                                        LysC#48----------

1101 TCTCAAACCTGTATGCACCTGAACACTTGATCATCAATGTCAAAGACGCTGAGAAATGGGAGGGACTGATAGAGAACGCAGGTTCGGTTTTCATAGGGCC  1200
       S  N  L  Y  A  P  E  H  L  I  I  N  V  K  D  A  E  K  W  E  G  L  I  E  N  A  G  S  V  F  I  G  P
     ----------(K)

1201 GTGGACTCCTGAGAGTGTTGGTGATTACGCGAGCGGGACAAACCACGTTCTTCCAACGTACGGGTATGCGAGAATGTACAGTGGCGTCTCTCTCGACTCT  1300
       W  T  P  E  S  V  G  D  Y  A  S  G  T  N  H  V  L  P  T  Y  G  Y  A  R  M  Y  S  G  V  S  L  D  S

1301 TTCCTGAAGTTCATGACTGTACAGTCCTTGACAGAGGAAGGTCTGCGAAACCTTGGTCCGTATGTTGCGACTATGGCTGAAATTGAAGGTCTAGATGCAC  1400
       F  L  K  F  M  T  V  Q  S  L  T  E  E  G  L  R  N  L  G  P  Y  V  A  T  M  A  E  I  E  G  L  D  A  H
     LysC#45----------
                                                                                CNBr#6----------

1401 ACAAGAGAGCCGTCACTCTCAGACTCAAGGATATCGAAGCCAAACAGACCCAAACAAAGTGAAATGAATGGCTGTCTTCATATTCAAATGAGGTGACATT  1500
       K  R  A  V  T  L  R  L  K  D  I  E  A  K  Q  T  Q  T  K  *
     ----------(?)----------

1501 GGTTTAAAGAGATAATAATAATAAGAATAATATAAGAGATGTTGTAACACTCTCTGTCATAATCTGGATTTTCTTTCATTATTGAAATTTGAATCCTTTC  1600

1601 ACGCGT  1606
```

FIG. 2. DNA sequence and deduced amino acid sequence of cDNA clone HDH7. Regions corresponding to the amino acid sequences of eight peptides determined from the purified protein are underlined. Residues from the peptides that do not match the translated cDNA sequence are indicated in parentheses. In cases where two ambiguous amino acids were detected, each is indicated, separated by a slash. Lysines or methionines, inferred from cleavage by lysyl endopeptidase (LysC) or CNBr, respectively, are included at the beginning of each peptide.

RESULTS AND DISCUSSION

HDH was purified to homogeneity by the method of Nagai and Scheidegger (26). The sequences of peptides from the purified enzyme were determined by automated Edman degradation and were found to have a high degree of similarity to the translated sequence of the yeast *HIS4C* gene, which encodes HDH (38). By assuming that the sequences of the plant and yeast enzymes were colinear, the relative positions of the peptides in the plant enzyme could be deduced by aligning them with the yeast sequence. Based on this analysis, degenerate oligonucleotide primers were synthesized that corresponded to two of the peptides in orientations appropriate for amplification of the plant cDNA (Fig. 1*A*). A polymerase chain reaction (39) was performed using DNA extracted from a phage λ cDNA library constructed from cabbage leaf tissue poly(A)$^+$ RNA. Primers predicted to lie approximately 1 kilobase (kb) apart generated several products detectable by ethidium bromide stain (Fig. 1*B*). One of these products was the expected length and hybridized specifically to a probe predicted to lie between the terminal primers (Fig. 1*C*). These data implied that the fragment contained a portion of the HDH cDNA, which was confirmed by DNA sequence analysis.

The 1-kb fragment from the polymerase chain reaction was purified, radiolabeled, and used to probe approximately 300,000 plaques from the same cDNA library. Fifteen strongly hybridizing clones were isolated and the size of their inserts was analyzed. Of several clones analyzed by partial DNA sequencing, one was found that appeared full-length. The complete DNA sequence and predicted amino acid sequence of this 1.6-kb clone are shown in Fig. 2.

The cDNA had an open reading frame of 469 codons, beginning at a methionine residue 31 codons upstream of the amino terminus of the mature protein coding sequence. The predicted mature protein was 438 amino acids long, having a calculated molecular weight of 47,474 and a calculated pI value of 5.6. These values are in reasonable agreement with measurements made on the purified protein that gave a molecular weight of 52,000 estimated by SDS/PAGE and a pI value of 5.1–5.4 estimated by isoelectric focusing (26). Comparison of the translated open reading frame to the sequences of eight peptides determined from the purified protein revealed matches at 151 out of 159 positions. It had previously been shown (26) that the purified protein preparation contains multiple electromorphs by isoelectric focusing gel electrophoresis that may correspond to multiple isozymes. The occasional mismatched residues between the protein sequence and the translated DNA sequence therefore may be due to the occurrence of multiple isozymes derived from distinct coding sequences.

Comparing the translated open reading frame to the amino-terminal protein sequence (Fig. 2) showed that HDH is encoded as a proenzyme, with a 31-residue amino-terminal peptide not present in the mature enzyme. The amino-terminal extension had a sequence composition typical of chloroplast transit peptides (40). Specifically, the peptide was rich in serine and threonine (32%) and in lysine and arginine (19%). Although the intracellular localization of histidine biosynthetic enzymes has not yet been determined, other amino acid biosynthetic pathways that have been studied in greater detail in plants have been found in the plastid compartment (41). The putative chloroplast transit peptide found on HDH, therefore, provides preliminary evidence that the histidine biosynthetic pathway in plants also is localized in the chloroplast.

The predicted mature protein sequence of the cabbage clone was 49% identical to the *E. coli* protein sequence and 51% identical to the sequence from *Saccharomyces cerevisiae*. Overall, the sequences were of approximately the same length and did not contain any apparent outstanding structural differences relative to each other (Fig. 3). Several regions, one of which was 15 amino acids long, were absolutely conserved across kingdoms. Interestingly, the cabbage sequence contained a cysteine codon at position 143 of the predicted protein coding sequence, which corresponds to an identical residue known to function in binding histidinol in the *Salmonella typhimurium* enzyme (42).

To test genetically whether the putative HDH cDNA clone could encode a functional HDH enzyme, we transformed an *E. coli* histidine auxotroph with a plasmid containing the cDNA. The bacterial strain WB353 lacks the entire *his* operon and can grow on minimal medium containing histidinol only if it harbors a functional HDH gene (43). Fig. 4 shows that WB353 harboring either the empty plasmid vector or a plasmid containing the cDNA clone could grow on

FIG. 3. Comparison among deduced HDH protein sequences from plant and microbial sources. Positions at which identical residues are present in three or more of the sequences are outlined. Amino acid residue numbers from the cabbage histidinol dehydrogenase predicted proenzyme sequence are indicated. The first amino acid in the *Saccharomyces cerevisiae* (*S. cerevisiae*) sequence shown corresponds to codon number 354 in the *HIS4* coding sequence (37). The first amino acids in the *E. coli* and *Salmonella typhimurium* (*S. typhimurium*) sequences correspond to codon 1 of the respective *hisD* coding sequences. A dot over the sequences marks the position of a cysteine implicated in binding histidinol in the *Salmonella typhiumurium* enzyme (42).

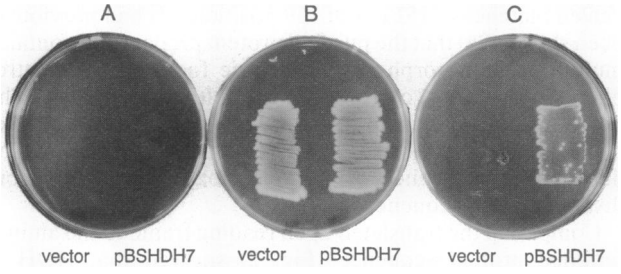

FIG. 4. Suppression of histidine auxotrophy in *E. coli* by the cabbage HDH. Cultures of strain WB353 (27) harboring either empty pBluescript (vector) or pBluescript bearing the HDH cDNA (pBSHDH7) were streaked onto M9 minimal glucose agar (44) containing ampicillin (100 μg/ml) and the following additives. (*A*) None. (*B*) L-Histidine (130 μM). (*C*) L-Histidinol (250 μM).

minimal medium containing histidine. In contrast, the vector-containing strain was completely unable to grow on histidinol, whereas the strain containing the plant HDH cDNA grew, although not as well as on medium containing histidine. In the pBluescript plasmid, the HDH cDNA lies downstream of the *lac* promoter, but its coding sequence is not in reading frame with the native *lacZ* initiation codon in the vector. Presumably, translation is able to initiate, albeit inefficiently, at sequences within the 5′ end of the cDNA, resulting in quantities of HDH sufficient to promote growth of the auxotroph on selective medium.

Northern blot analysis was performed on poly $(A)^+$ RNA from cabbage leaf tissue to determine the size of the HDH transcript. Fig. 5*A* shows that the cDNA probe detected a single mRNA approximately 1.6 kb in length. This result indicates that the clone is at least very close to a full-length representation of the mRNA.

To estimate the number of genes for HDH present in the cabbage genome, we digested cabbage DNA with various restriction enzymes that lacked recognition sites within the cDNA clone, separated the fragments by agarose gel electrophoresis, and blotted the gel to nylon membrane (35). The cDNA probe hybridized to more than one genomic fragment (Fig. 5*B*), indicating that the cabbage genome may contain multiple genes for HDH. This finding is consistent with the possibility that cabbage contains multiple HDH isozymes encoded by different genes. Whether these genes are differentially expressed can now be addressed using the cDNA clone.

Several lines of evidence indicate that the clone we describe here encodes HDH. (*i*) The predicted amino acid sequence is in close agreement with the sequence of peptides from the purified protein. (*ii*) The sequence of the predicted mature protein encoded by the clone is homologous to the yeast and bacterial sequences over its entire length. (*iii*) The clone could complement an *E. coli his* deletion mutant in the presence of the substrate histidinol. Thus, the plant HDH appears to be conserved structurally as well as functionally compared to the corresponding enzymes from prokaryotic and eukaryotic microbes.

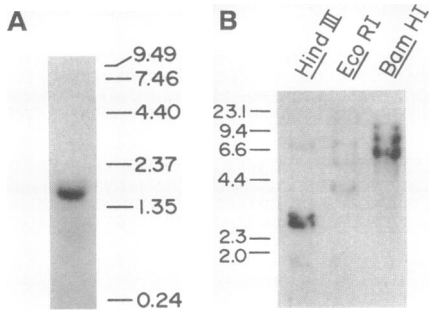

FIG. 5. Analysis of the expression and genomic complexity of HDH in cabbage. (*A*) Northern blot analysis of poly$(A)^+$ RNA extracted from mature cabbage leaves. The positions of RNA size markers (in kb) are indicated. (*B*) Southern blot analysis of cabbage genomic DNA digested with the enzymes indicated and probed with the cDNA insert from plasmid subclone pBSHDH7. The positions of DNA size markers (in kb pairs) are indicated.

For excellent technical assistance, we thank Rene Knecht. We gratefully acknowledge Wayne Barnes for providing bacterial strains. We also thank our colleagues Mary-Dell Chilton, Kay Lawton, Jim Ligon, and Scott Uknes for critically reading the manuscript.

1. Stewart, G. R., Mann, A. F. & Fentem, P. A. (1980) in *The Biochemistry of Plants*, ed. Miflin, B. J. (Academic, New York), Vol. 5, pp. 271–327.
2. Suzuki, A. & Gadal, P. (1984) *Physiol. Veg.* **22,** 471–486.
3. Srivastava, H. S. & Singh, R. P. (1987) *Phytochemistry* **26,** 597–610.
4. Relton, J. M., Bonner, P. L. R., Wallsgrove, R. M. & Lea, P. J. (1988) *Biochim. Biophys. Acta* **953,** 48–60.
5. Krishnaswamy, S. & Bryan, J. K. (1986) *Arch. Biochem. Biophys.* **246,** 250–262.
6. Kumpaisal, R., Hashimoto, T. & Yamada, Y. (1987) *Plant Physiol.* **85,** 145–151.
7. Suzich, J. A., Dean, J. F. D. & Herrmann, K. M. (1985) *Plant Physiol.* **79,** 765–770.
8. Shah, D. M., Horsch, R. B., Klee, H. J., Kishore, G. M., Winter, J. A., Tumer, N. E., Hironaka, C. M., Sanders, P. R., Gasser, C. S., Aykent, S., Siegel, N., Rogers, S. G. & Fraley, R. T. (1986) *Science* **233,** 478–481.
9. Cullimore, J. V., Gebhardt, C., Saarelainen, R., Miflin, B. J., Idler, K. B. & Barker, R. F. (1984) *J. Mol. Appl. Genet.* **2,** 589–599.
10. Dyer, W. E., Weaver, L. M., Zhao, J., Kuhn, D. N., Weller, S. C. & Herrmann, K. M. (1990) *J. Biol. Chem.* **265,** 1608–1614.
11. Kaneko, T., Hashimoto, T., Kumpaisal, R. & Yamada, Y. (1990) *J. Biol. Chem.* **265,** 17451–17455.
12. Matthews, B. F., Reardon, E. M., Turano, F. J. & Wilson, B. J. (1988) *Plant Mol. Biol. Rep.* **6,** 137–154.
13. Mazur, B. J., Chui, C.-F. & Smith, J. K. (1987) *Plant Physiol.* **85,** 1110–1117.
14. Tingey, S. V. & Coruzzi, G. M. (1987) *Plant Physiol.* **84,** 366–373.
15. Berlyn, M. B., Last, R. L. & Fink, G. R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4604–4608.
16. Snustad, D. P., Hunsperger, J. P., Chereskin, B. M. & Messing, J. (1988) *Genetics* **120,** 1111–1124.
17. Delauney, A. J. & Verma, D. P. S. (1990) *Mol. Gen. Genet.* **221,** 299–305.
18. Ahmed, A., Case, M. E. & Giles, N. H. (1964) *Brookhaven Symp. Biol.* **17,** 53–65.
19. Fink, G. R. (1964) *Science* **146,** 525–527.
20. Burke, M. E. & Pattee, P. A. (1972) *Can. J. Microbiol.* **18,** 569–576.
21. Kane-Falce, C. & Kloos, W. E. (1975) *Genetics* **79,** 361–376.
22. Winkler, M. E. (1987) in *Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington), pp. 395–411.
23. Miflin, B. J. (1980) in *The Biochemistry of Plants*, ed. Miflin, B. J. (Academic, New York), Vol. 5, pp. 533–539.
24. Davies, M. E. (1971) *Phytochemistry* **10,** 783–788.
25. Wiater, A., Krajewska-Grynkiewicz, K. & Kloptowski, T. (1971) *Acta Biochim. Pol.* **18,** 299–307.
26. Nagai, A. & Scheidegger, A. (1991) *Arch. Biochem. Biophys.* **284,** 127–132.
27. Smith, G. R. & Tong, B. (1974) *J. Bacteriol.* **120,** 1223–1226.
28. Barnes, W. M. & Tuley, E. (1983) *J. Mol. Biol.* **165,** 443–459.
29. Strickler, J. E., Hunkapiller, M. W. & Wilson, K. J. (1984) *Anal. Biochem.* **140,** 553–566.
30. Lagrimini, L. M., Burkhart, W., Moyer, M. & Rothstein, S. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7542–7546.

31. Hattoni, M. & Sakaki, Y. (1986) *Anal. Biochem.* **152,** 232–238.
32. Devereaux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
33. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1983) *Current Protocols in Molecular Biology* (Wiley, New York).
34. Payne, G., Ward, E., Gaffney, T., Ahl-Goy, P., Moyer, M., Harper, A., Meins, F. Jr., & Ryals, J. (1990) *Plant Mol. Biol.* **15,** 797–808.
35. Reed, K. C. & Mann, D. A. (1985) *Nucleic Acids Res.* **13,** 7207–7221.
36. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
37. Marchionni, M. & Gilbert, W. (1986) *Cell* **46,** 133–141.
38. Donahue, T. F., Farabaugh, P. J. & Fink, G. R. (1982) *Gene* **18,** 47–59.
39. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
40. Schmidt, G. W. & Mishkind, M. L. (1986) *Annu. Rev. Biochem.* **55,** 879–912.
41. Miflin, B. J., ed. (1980) *The Biochemistry of Plants* (Academic, New York), Vol. 5.
42. Grubmeyer, C. T. & Gray, W. R. (1986) *Biochemistry* **25,** 4778–4784.
43. Barnes, W. M. (1981) *J. Bacteriol.* **147,** 124–134.
44. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), p. 431.