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Molecular cloning of a plant betaine-aldehyde dehydrogenase, an enzyme implicated in adaptation to salinity and drought

(oligonucleotide screening/amino acid sequence/cDNA clone/spinach/transit peptide)

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ABSTRACT **Many plants, as well as other organisms, accumulate betaine (*N,N,N*-trimethylglycine) as a nontoxic or protective osmolyte under saline or dry conditions. In plants, the last step in betaine synthesis is catalyzed by betaine-aldehyde dehydrogenase (BADH, EC 1.2.1.8), a nuclear-encoded chloroplastic enzyme. A cDNA clone for BADH (1812 base pairs) was selected from a λgt10 cDNA library derived from leaves of salt-stressed spinach (*Spinacia oleracea* L.). The library was screened with oligonucleotide probes corresponding to amino acid sequences of two peptides prepared from purified BADH. The authenticity of the clone was confirmed by nucleotide sequence analysis; this analysis demonstrated the presence of a 1491-base-pair open reading frame that contained sequences encoding 12 peptide fragments of BADH. The clone hybridized to a 1.9-kilobase mRNA from spinach leaves; this mRNA was more abundant in salt-stressed plants, consistent with the known salt induction of BADH activity. The amino acid sequence deduced from the BADH cDNA sequence showed substantial similarities to those for nonspecific aldehyde dehydrogenases (EC 1.2.1.3 and EC 1.2.1.5) from several sources, including absolute conservation of a decapeptide in the probable active site. Comparison of deduced and determined amino acid sequences indicated that the transit peptide may comprise only 7 or 8 residues, which is atypically short for precursors to stromal proteins.**

Higher plants from several families (e.g., Chenopodiaceae, Poaceae, Asteraceae) accumulate the quaternary ammonium compound betaine in response to salt stress or water deficit (1). Much evidence indicates that in plants and in other organisms, betaine acts as a nontoxic or protective cytoplasmic osmolyte, allowing normal metabolic function to continue in cells at low solute potential (2–4). These findings have focused interest on the betaine synthesis pathway and the corresponding genes, in relation to the regulatory mechanisms involved and to possible genetic engineering of stress resistance (5).

Betaine is derived in plants, as in other organisms, by a two-step oxidation of choline (1). Both of these steps occur in the chloroplast (6).

$$\underset{\text{Choline}}{(CH_3)_3\overset{+}{N}CH_2CH_2OH} \rightarrow \underset{\text{Betaine aldehyde}}{(CH_3)_3\overset{+}{N}CH_2CHO} \rightarrow \underset{\text{Betaine}}{(CH_3)_3\overset{+}{N}CH_2COO^-}$$

The second step is catalyzed by a betaine-aldehyde dehydrogenase (BADH, EC 1.2.1.8) that appears to be substrate-specific and to prefer NAD^+ as electron acceptor (7–10). In spinach (*Spinacia oleracea* L.) leaves, ≈90% of the BADH activity is localized in the chloroplast stroma, with the remainder apparently in a minor cytosolic isozyme (10, 11). The stromal enzyme has been purified to homogeneity (7, 12); it is a dimer of identical subunits of M_r 60,000–63,000, encoded by a single nuclear gene (11). Consistent with causing accumulation of betaine in salinized plants, salt stress increases the levels of BADH activity (10, 13), BADH protein, and translatable BADH message in spinach (12). To investigate this stress-induction process further, and as a step toward molecular genetic manipulation of the betaine pathway in plants, we have isolated cDNA clones encoding spinach BADH. The deduced amino acid sequence shows that BADH is structurally related to nonspecific aldehyde dehydrogenases (ALDH), and indicates that the transit peptide is unusually short.§

MATERIALS AND METHODS

Plant Material. Spinach (*S. oleracea* L.) cv. Savoy Hybrid 612 (Harris Moran Seeds, Rochester, NY) was used for all experiments; this cultivar carries the slow (S) allele of BADH (11). Plant growth conditions were as given previously (11). For salinization treatments, NaCl concentration was raised gradually to a final concentration of 200 mM, as described (10). Total RNA and poly(A)$^+$ RNA were isolated from leaves of control and salinized plants (12). Salinization did not affect the yields of total RNA and poly(A)$^+$ RNA. The latter was not contaminated with ribosomal RNA, as judged by ethidium bromide staining following electrophoretic separation.

Amino Acid Sequence Analyses. BADH was purified to homogeneity (12) and desalted by reverse-phase HPLC using an Aquapore phenyl column (2.1 × 30 mm; Brownlee Laboratory) eluted with a linear 0–90% gradient of acetonitrile/2-propanol, 1:1 (vol/vol), in 0.1% trifluoroacetic acid. For reduction and alkylation, the protein was incubated in 6 M guanidine hydrochloride/1 M Tris·HCl, pH 8.6/10 mM EDTA/20 mM dithiothreitol for 1 hr at 37°C (14); 4-vinylpyridine was then added to 50 mM, and the incubation continued for 1 hr at room temperature (15). The modified protein was desalted as above. No sequence was obtained when 125 pmol of intact, modified protein was subjected to Edman degradation, indicating that the amino terminus was blocked. The modified protein was digested with trypsin or endoproteinase Lys-C (Boehringer Mannheim). Peptides were isolated by reverse-phase HPLC using a Hypersil-ODS column (2.1 × 100 mm; Hewlett–Packard) eluted with a linear 0–60% gradient of acetonitrile/2-propanol, 1:1 (vol/vol), in 0.1% trifluoroacetic acid. Amino-terminal sequences of 12 peptides were determined by automated Edman degradation with an Applied Biosystems 470A gas-phase sequencer.

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Abbreviations: ALDH, aldehyde dehydrogenase; BADH, betaine-aldehyde dehydrogenase.

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§The sequence reported in this paper has been deposited in the EMBL/GenBank data base (accession no. M31480).

Phenylthiohydantoin derivatives of amino acids were identified using an Applied Biosystems 120A PTH analyzer.

Selection of Oligodeoxynucleotide Probes. From the amino acid sequences determined for BADH peptides, two were chosen to generate mixed 20-mer oligonucleotide probes with minimal degeneracy. The sequence Asp-Pro-Phe-Glu-Glu-Gly-Cys was used to model probe 1, 5'-CANCCYTCYTC(A or G)AANGGRTC-3'; Phe-Glu-Asp-Val-Asp-Ile-Asp was used for probe 2, 5'-TCDATRTCNACRTC(T or C)TCRAA-3'. Each probe was synthesized as two subpools (probes 1A, 1G and 2T, 2C) with alternative nucleotides at one position, as indicated. Probe degeneracy was 128-fold for 1A and 1G, 96-fold for 2T and 2C. RNA blot analysis was used to select one subpool from each pair for use in screening the cDNA library. Poly(A)$^+$ RNA from control and salinized plants was denatured and electrophoresed (2 μg per lane) in 1.5% agarose gels with 1.0 M formaldehyde, transferred to nitrocellulose, and hybridized with probes 1A, 1G, 2T, and 2C. Probe labeling with ^{32}P, filter washing, and autoradiography were essentially as given below.

Isolation of cDNA Clones. Poly(A)$^+$ RNA prepared from leaves of salinized spinach plants was used to construct a λgt10 cDNA library using the RiboClone system (Promega) with oligo(dT) as primer and *Eco*RI adaptors, as described in the Promega Protocols and Applications Guide. The library was not amplified. Approximately 240,000 recombinant phage were plated at a density of 30,000 plaques per 15-cm plate. Duplicate nitrocellulose filter lifts were made and hybridized with oligonucleotide probes 1A and 2T. The probes were end-labeled with [γ-^{32}P]ATP to a specific activity of $\approx 10^7$ cpm/pmol by polynucleotide kinase. Hybridizations were carried out at a concentration of 10^7 cpm/ml of 5× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0)/0.2% Ficoll/0.2% polyvinylpyrrolidone/0.2% bovine serum albumin/50 mM sodium phosphate buffer, pH 7.0/0.1% SDS with brewer's yeast tRNA (100 μg/ml), at 51°C for probe 1A and 45°C for probe 2T. Filters were washed twice in 5× SSC/0.1% SDS/0.1% sodium pyrophosphate, once at 30°C and once at either 46°C (probe 1A) or 40°C (probe 2T), and examined by autoradiography at −70°C with an intensifying screen.

A single clone that hybridized to both oligonucleotide probes was detected and purified. A 0.64-kilobase (kb) *Eco*RI fragment from the cDNA insert of this clone was subcloned into plasmid pUC119. For use in rescreening the cDNA library, the subcloned 0.64-kb fragment was excised, purified by agarose gel electrophoresis, and labeled by random-primed incorporation of digoxigenin-labeled dUTP with a commercial kit (Boehringer Mannheim). An additional 200,000 recombinant phage were screened, following manufacturer's protocols for hybridization and immunological detection. One positive clone was detected; this clone was purified and the DNA was isolated for further analyses.

DNA Sequence Analysis. A partial restriction map and subcloning strategy for the cDNA insert of the clone obtained by oligonucleotide screening are shown in Fig. 1. The 0.64-kb *Eco*RI fragment was inserted into pUC119 in both orientations. The remainder of the insert could not be excised from the vector because of loss of one of the flanking *Eco*RI sites. The remainder of the insert was therefore cloned into pUC118 and pUC119 as a 2.1-kb *Eco*RI–*Bgl* II fragment that included 0.9 kb of λgt10 DNA. To confirm the orientation of the 0.64-kb *Eco*RI fragment, a *Hin*dIII fragment that included the 0.64-kb *Eco*RI fragment and 0.2 kb of flanking λgt10 DNA was cloned into pUC119 in both orientations. Single-stranded plasmid DNA was prepared (16), and deletion subclones were generated (17). Single-stranded deletion subclones were sequenced by the dideoxynucleotide chain-termination method (18). A similar subcloning strategy was used to completely sequence the insert (1.8 kb) of the clone isolated by using the 0.64-kb *Eco*RI fragment as a probe. DNA sequence analysis indicated that the BADH insert of this second clone was derived from the same mRNA species as the first.

RNA Blot Analysis. Poly(A)$^+$ RNA isolated from control and salinized spinach plants was denatured, subjected to electrophoresis (2 μg per lane) in formaldehyde/1.5% agarose gels (19), and transferred to nitrocellulose. Filters were hybridized with the 0.64-kb *Eco*RI restriction fragment labeled with ^{32}P by the random primer method (20). After autoradiography, radiolabeled bands from RNA gel blots were cut out, and the ^{32}P activity was assayed by scintillation counting.

RESULTS

Isolation and Characterization of a BADH cDNA Clone. When the two pairs of oligonucleotide probes were tested by blot hybridization analysis against poly(A)$^+$ RNA from control and salinized plants, all four probes hybridized to several discrete mRNA species. However, only probes 1A and 2T hybridized to an ≈1.9-kb mRNA species whose level was higher in salinized plants (data not shown). Because this size and salt-inducibility are consistent with expectations for BADH mRNA (12), these two probes were used to screen the cDNA library.

From 240,000 independent transformants screened, only one clone was identified that hybridized with both probes. This clone contained an 1812-base-pair (bp) cDNA insert (Fig. 2) with a single long open reading frame beginning at nucleotide 62 and terminating at nucleotide 1558. Initiation of protein coding is likely to begin at the ATG codon of the sequence AAGAATGGC. This sequence, which starts at nucleotide position 64, strongly resembles the consensus sequence AACAATGGC that surrounds the ATG initiation codon in plants (21). Therefore, the sequence of the cDNA insert contains 67 nucleotides of the 5' noncoding region, a coding region of 1491 nucleotides, and 239 nucleotides of a 3' noncoding region. The 3' noncoding region includes a putative polyadenylylation signal (AATAAA) 120 nucleotides upstream from the poly(A) tail (Fig. 2). Any ambiguities in the sequence of this cDNA insert were resolved by reference to the DNA sequence of the second, independent λgt10 isolate (see *Materials and Methods*).

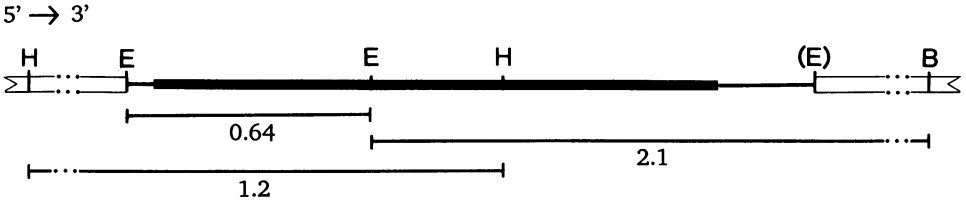

FIG. 1. Partial restriction map and subcloning strategy for spinach BADH cDNA. Heavy lines indicate the protein-coding region; open boxes are λ vector DNA, which is not shown to scale. Restriction endonuclease cleavage sites: E, *Eco*RI; (E), missing *Eco*RI site; H, *Hin*dIII; B, *Bgl* II. Fragment sizes are in kilobases.

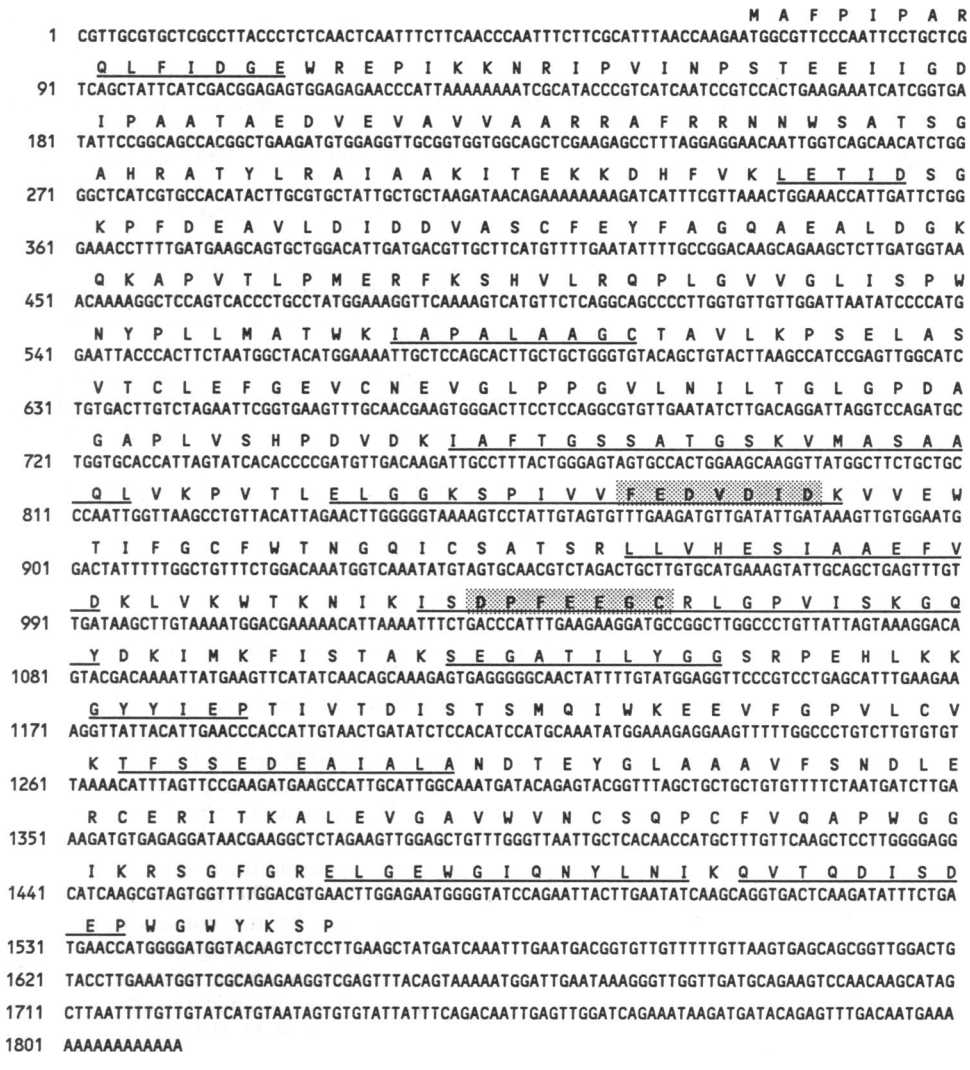

FIG. 2. Nucleotide sequence of spinach BADH cDNA, and the deduced amino acid sequence. The two amino acid sequences used to model oligonucleotide probes are shaded. Amino acid sequences determined for peptide fragments of mature BADH are underlined. For the tryptic peptide corresponding to nucleotides 1466–1504, direct sequence data were ambiguous at residue 5, indicating both tyrosine and tryptophan.

Deduced Primary Structure of Spinach BADH. The single open reading frame of the cloned cDNA encodes 497 amino acid residues (Fig. 2). The deduced amino acid sequence contains segments matching the sequences determined for 12 peptide fragments of purified BADH (Fig. 2). In addition, the nucleotide sequence at positions 827–856 encodes the decapeptide Val-Thr-Leu-Glu-Leu-Gly-Gly-Lys-Ser-Pro (Fig. 3), which is highly conserved among general ALDHs (22). The calculated molecular weight of the 497-residue polypeptide is 54,267. This value is typical of ALDH subunits (22), and in good agreement with the M_r of 111,000 measured for the native BADH dimer by nondenaturing gel permeation chromatography (12). The estimates of 60,000 (7) or 63,000 (12) obtained for the BADH monomer by SDS/PAGE are clearly higher. However, the magnitude of the discrepancy for BADH lies in a range attributable to the influence of the primary structure of a particular protein on the amount of SDS it binds (30).

The deduced BADH amino acid sequence was compared with deduced or determined amino acid sequences for the ALDHs listed in Fig. 3, using the PROSIS computer program (Hitachi). Comparisons made for successive 200-residue re-

FIG. 3. Presence in spinach BADH of the same decapeptide sequence and cysteine residue as occur in nonspecific ALDHs from microbial and mammalian sources. The valine at the start of the decapeptide corresponds to the following residues for the various enzymes: spinach BADH, 254; *A. nidulans aldA* gene product, 273; *P. oleovorans alkH* gene product, 223; all others, 265. References for sequence data (in the order *A. nidulans aldA* → bovine mitochondrial ALDH) were 23, 24, 25, 26, 27, 27, 28, 29, 26.

gions along the proteins demonstrated amino acid identities of 29–48%, with the central one-third of the protein typically showing a higher value than the remainder. Overall amino acid identities between the entire BADH protein and various ALDHs were lowest for *P. oleovorans* and rat hepatoma ALDHs (31% and 30%, respectively) and highest for *A. nidulans* and horse cytosolic enzymes (42%).

Expression of BADH mRNA. RNA blot hybridization of poly(A)$^+$ RNA isolated from control or salinized spinach leaves showed a single transcript of ≈1.9 kb (Fig. 4). The signal was 2-fold greater in the sample from salinized tissue, indicating that BADH mRNA accumulates in response to salt stress. This is consistent with the increased level of translatable BADH message in salinized spinach leaves (12) and the accompanying rises in BADH enzyme activity (10, 13) and BADH protein (12).

DISCUSSION

The matches between deduced and directly determined amino acid sequences, distributed throughout the open reading frame, confirm the authenticity of the BADH cDNA clone reported in Fig. 2. The cDNA insert approaches the size of the BADH message (1812 bp and ≈1.9 kb, respectively), indicating that the clone is near full-length. This inference is supported by the presence of 5′ and 3′ noncoding regions, the latter terminating in a poly(A) tail.

The major spinach BADH isozyme is located in the chloroplast stroma (10) and is encoded by a nuclear gene (11). As for other nuclear-encoded chloroplast proteins, stromal BADH is thus expected to be synthesized as a larger precursor containing an amino-terminal transit peptide (31). Earlier experiments with *in vitro* translation of BADH mRNA showed that the primary translation product of BADH is indeed larger than the mature peptide, but only by about 1.2 kDa (12). Assuming that the mature peptide is not posttranslationally modified, this result predicted a transit peptide of ≤10 amino acid residues, which is exceptionally short for a protein imported into the chloroplast (31). The deduced primary structure of BADH is consistent with this surprising prediction, because there is a match between the deduced amino acid sequence and the heptapeptide fragment Gln-Leu-Phe-Ile-Asp-Gly-Glu only 8 amino acids after the predicted ATG initiation codon. The heptapeptide was generated by tryptic digestion of intact BADH protein, which has a blocked amino terminus. Cleavage would have occurred between glutamine and the preceding arginine residue had the α-amino group of the latter been acylated. An economical interpretation of the above data is that the transit peptide of BADH comprises the short fragment Met-Ala-Phe-Pro-Ile-Pro-Ala and that the amino terminus of the processed stromal BADH monomer is a blocked arginine residue. Were this so, the BADH monomer would have a calculated molecular weight close to 53,540.

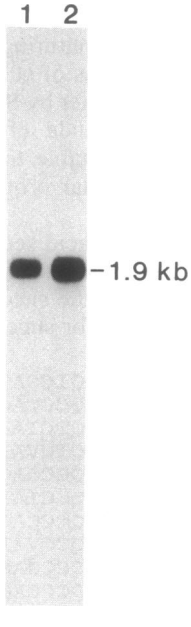

FIG. 4. RNA gel blot analysis of BADH mRNA. Poly(A)$^+$ RNA (2 μg) isolated from leaves of control (lane 1) and salinized (lane 2) spinach plants was electrophoresed, transferred to nitrocellulose, and probed with the 0.64-kb *Eco*RI restriction fragment shown in Fig. 1.

Specific BADHs have been identified in microorganisms (32, 33) and mammalian liver (34), but amino acid sequences are not available either for these or for other substrate-specific ALDHs. It has thus not yet been established whether such specialized ALDHs are structurally related to ALDHs of broad substrate specificity (EC 1.2.1.3 and 1.2.1.5), or whether they are distinct (35). It is therefore interesting that our comparisons between spinach BADH and a variety of nonspecific ALDHs demonstrated structural similarities consistent with a common evolutionary origin, despite differences in their physical and functional properties. Thus, BADH is a dimer; it has little or no activity against small aliphatic or aromatic aldehydes, and prefers NAD$^+$ to NADP$^+$ (8, 10). On the other hand, mammalian ALDHs are generally tetramers that oxidize a range of aliphatic aldehydes using NAD$^+$ as coenzyme, although the rat hepatoma ALDH is a dimer that prefers aromatic aldehyde substrates and uses NADP$^+$ (22, 25); the *A. nidulans* ALDH oxidizes acetaldehyde and other small aldehydes using either NAD$^+$ or NADP$^+$ (36). The case for an evolutionary relationship is strengthened by the conservation of the decapeptide and cysteine residue highlighted in Fig. 3, and by the siting of these motifs in similar positions in BADH and the various ALDHs. The glutamic residue of the decapeptide and the cysteine located 34 residues from it have both been implicated in catalysis in the mammalian enzymes (22). Sequences around the cysteine residue have been proposed to play a role in substrate specificity (22); the BADH primary structure will help identify target residues for modification by site-directed mutagenesis in tests of this idea.

The rise in the level of BADH mRNA in salinized plants could result from stress-enhanced transcription or greater mRNA stability; there are precedents for both these mechanisms from plant responses to other physicochemical stresses (37, 38). Whatever its cause, the increase in BADH mRNA establishes that stress-induced betaine accumulation in plants involves, and may require, modulation of the expression of a betaine biosynthesis gene. Such links between salt- or water-stress metabolism and gene expression have rarely been demonstrated for plants: many osmotic stress-induced plant genes have been reported (e.g., refs. 39 and 40), but very few correspond to known proteins whose biochemical or physiological function is apparent (41, 42).

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1. Wyn Jones, R. G. & Storey, R. (1981) in *The Physiology and Biochemistry of Drought Resistance in Plants*, eds. Paleg,

L. G. & Aspinall, D. (Academic, Sydney, Australia), pp. 171–204.
2. Schroppel-Meier, G. & Kaiser, W. M. (1988) *Plant Physiol.* **87,** 822–827.
3. Somero, G. N. (1986) *Am. J. Physiol.* **251,** R197–R213.
4. Wyn Jones, R. G. (1984) *Recent Adv. Phytochem.* **18,** 55–78.
5. Le Rudulier, D., Strom, A. R., Dandekar, A. M., Smith, L. T. & Valentine, R. C. (1984) *Science* **224,** 1064–1068.
6. Hanson, A. D., May, A. M., Grumet, R., Bode, J., Jamieson, G. C. & Rhodes, D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3678–3682.
7. Arakawa, K., Takabe, T., Sugiyama, T. & Akazawa, T. (1987) *J. Biochem. (Tokyo)* **101,** 1485–1488.
8. Pan, S.-M., Moreau, R. A., Yu, C. & Huang, A. H. C. (1981) *Plant Physiol.* **67,** 1105–1108.
9. Weretilnyk, E. A., Bednarek, S., McCue, K. F., Rhodes, D. & Hanson, A. D. (1989) *Planta* **178,** 342–352.
10. Weigel, P., Weretilnyk, E. A. & Hanson, A. D. (1986) *Plant Physiol.* **82,** 753–759.
11. Weretilnyk, E. A. & Hanson, A. D. (1988) *Biochem. Genet.* **26,** 143–151.
12. Weretilnyk, E. A. & Hanson, A. D. (1989) *Arch. Biochem. Biophys.* **271,** 56–63.
13. Pan, S.-M. (1983) *Taiwania* **28,** 128–137.
14. Allen, G. (1981) in *Sequencing of Proteins and Peptides: Laboratory Techniques in Biochemistry and Molecular Biology*, eds. Work, T. S. & Burdon, R. H. (Elsevier, Amsterdam), Vol. 9.
15. Andrews, P. C. & Dixon, J. E. (1987) *Anal. Biochem.* **161,** 1–5.
16. Vieira, J. & Messing, J. (1987) *Methods Enzymol.* **153,** 3–11.
17. Dale, R. M. K. & Arrow, A. (1987) *Methods Enzymol.* **155,** 204–214.
18. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
19. Hershey, H. P., Colbert, J. T., Lissemore, J. L., Barker, R. F. & Quail, P. H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2332–2336.
20. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
21. Lutcke, H. A., Chow, K. C., Mickel, F. S., Moss, K. A., Kern, H. F. & Scheele, G. A. (1987) *EMBO J.* **6,** 43–48.
22. Pietruszko, R. (1989) in *Biochemistry and Physiology of Substance Abuse*, ed. Watson, R. A. (CRC, Boca Raton, FL), Vol. 1, pp. 89–127.
23. Pickett, M., Gwynne, D. I., Buxton, F. P., Elliott, R., Davies, R. W., Lockington, R. W., Scazzocchio, C. & Sealy-Lewis, H. M. (1987) *Gene* **51,** 217–226.
24. Kok, M., Oldenhuis, R., van der Linden, M. P. G., Meulenberg, C. H. C., Kingma, J. & Witholt, B. (1989) *J. Biol. Chem.* **264,** 5442–5451.
25. Jones, D. E., Brennan, M. D., Hempel, J. & Lindahl, R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1782–1786.
26. Farres, J., Guan, K.-L. & Weiner, H. (1989) *Eur. J. Biochem.* **180,** 67–74.
27. Hsu, L. C., Tani, K., Fujiyoshi, T., Kurachi, K. & Yoshida, A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3771–3775.
28. von Bahr-Lindstrom, H., Hempel, J. & Jornvall, H. (1984) *Eur. J. Biochem.* **141,** 37–42.
29. Johansson, J., von Bahr-Lindstrom, H., Jeck, R., Woenckhaus, C. & Jornvall, H. (1988) *Eur. J. Biochem.* **172,** 527–533.
30. de Jong, W. W., Zweers, A. & Cohen, L. H. (1978) *Biochem. Biophys. Res. Commun.* **82,** 532–539.
31. Keegstra, K., Olsen, L. J. & Theg, S. M. (1989) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40,** 471–501.
32. Mori, N., Kawakami, B., Hyakutome, K., Tani, Y. & Yamada, H. (1980) *Agric. Biol. Chem.* **44,** 3015–3016.
33. Landfald, B. & Strom, A. R. (1986) *J. Bacteriol.* **165,** 849–855.
34. Goldberg, A. M. & McCaman, R. E. (1968) *Biochim. Biophys. Acta* **167,** 186–189.
35. Weiner, H. (1989) in *Enzymology and Molecular Biology of Carbonyl Metabolism*, eds. Weiner, H. & Flynn, T. G. (Liss, New York), Vol. 2, pp. xix–xxi.
36. Pateman, J. A., Doy, C. H., Olsen, J. E., Norris, U., Creaser, E. H. & Hynes, M. (1983) *Proc. R. Soc. London Ser. B* **217,** 243–264.
37. Walker, J. C., Howard, E. A., Dennis, E. S. & Peacock, W. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6624–6628.
38. Beach, L. R., Spencer, D., Randall, P. J. & Higgins, T. J. V. (1985) *Nucleic Acids Res.* **13,** 999–1013.
39. Singh, N. K., Nelson, D. E., Kuhn, D., Hasegawa, P. M. & Bressan, R. A. (1989) *Plant Physiol.* **90,** 1096–1101.
40. Mundy, J. & Chua, N.-H. (1988) *EMBO J.* **7,** 2279–2286.
41. Michalowski, C. B., Olson, S. W., Piepenbrock, M., Schmitt, J. & Bohnert H. J. (1989) *Plant Physiol.* **89,** 811–816.
42. Delauney, A. J. & Verma, D. P. S. (1990) *Mol. Gen. Genet.*, in press.