The EMBO Journal vol.5 no.3 pp.451–458, 1986

The structure and expression of maize genes encoding the major heat shock protein, hsp70

Dean E.Rochester, Jill A.Winer and Dilip M.Shah

Division of Biological Sciences, Corporate Research and Development, Monsanto Company, St. Louis, MO 63198, USA

Communicated by J.Schell

We have isolated and sequenced two maize genomic clones that are homologous to the *Drosophila hsp70* gene. One of the maize *hsp70* clones contains the entire hsp70 coding region and 81 nucleotides of the 5′ nontranslated sequence. The predicted amino acid sequence for this maize protein is 68% homologous to the hsp70 of *Drosophila*. The second maize *hsp70* clone contains only part of the coding sequence and 1.1 kb of the 5′ flanking sequence. This 5′ flanking sequence contains two sequences homologous to the consensus heat-shock-element sequence. Both maize genes are thermally inducible and each contains an intron in the same position as that of the heat-shock-cognate gene, *hsc1*, of *Drosophila*. The presence of an intron in the maize genes is a distinguishing feature in that no other thermally inducible *hsp70* genes described to date contain an intron. We have constructed a hybrid *hsp70* gene containing the entire hsp70 coding sequence with an intron, and 1.1 kb of the 5′ flanking sequence. We demonstrate that this hybrid gene is thermally inducible in a transgenic petunia plant and that the gene is expressed from its own promoter.

Key words: heat shock genes/maize/plant transformation

Introduction

In higher plants, thermal stress induces the synthesis of a set of heat-shock proteins (hsps) ranging in mol. wt. from 15 to 110 kd (Kimpel and Key, 1985). This induction occurs at the transcriptional level and is similar to the response observed in bacteria, fungi, insects and mammals (Craig, 1985). These hsp mRNAs translate efficiently at high temperatures, resulting in a rapid accumulation of hsps in the stressed plant cells (Lin *et al.*, 1984).

In plants the small mol. wt. hsps are abundant (Lin *et al.*, 1984). Although the exact functions of the plant hsps remain unidentified, it is known that at least one hsp (hsp22) is transported into the chloroplast during heat shock (Koppstech *et al.*, 1985; Vierling *et al.*, 1986). The larger mol. wt hsps, also highly expressed, have not been as well characterized in plants as in other systems. One of these hsps, hsp70, is synthesized in response to heat stress in all organisms examined to date and its sequence is highly conserved among these diverse organisms (Craig, 1985). In *Drosophila* and in yeast the *hsp70* genes have been studied in depth (Craig, 1985). These genes are encoded by small families, the members of which are classified according to thermal inducibility. One class of these genes, the cognates, is expressed constitutively, and their expression is not enhanced by heat shock. Transcription of the other classes of *hsp70* genes is heat inucible (Craig *et al.*, 1982).

We have previously described the isolation and partial characterization of a maize *hsp70* genomic clone (Shah *et al.*, 1985). In this paper we report the sequence and expression of two maize *hsp70* genes. The homology between the predicted amino acid sequence of maize hsp70 and hsp70s from other organisms indicates that this protein has been highly conserved throughout evolution. We find that the maize *hsp70* genes are thermally inducible yet, due to the presence of an intron, they are structurally similar to the *Drosophila* heat-shock-cognate gene, *hsc1*. In addition we show that a hybrid maize *hsp70* gene is thermally inducible in a transgenic petunia plant.

Results

Sequence analysis of the maize hsp70 genes

The nucleotide sequence of the λM1 maize *hsp70* gene subcloned in pMON9502 (see Figure 1A) is shown in Figure 2. This sequence corresponds to the entire maize hsp70 polypeptide, 81 nucleotides of the 5′ nontranslated region and 66 nucleotides of the 3′ nontranslated region. The coding sequence of this maize gene is interrupted by a 0.7-kb intron which splits the codon specifying aspartic acid at position 71. The donor and acceptor splice junction sequences for this intron are in agreement with the consensus sequences for such junctions. The predicted amino acid sequence of the maize hsp70 polypeptide consists of 646 amino acids. When compared with the *Drosophila* hsp70, the maize polypeptide contains two single and one double amino acid insertions as well as three single and one double amino acid deletions (Figure 3). The predicted amino terminus of the maize hsp70 protein has five additional amino acids when compared with the amino terminal sequence of *Drosophila* hsp70. This type of amino

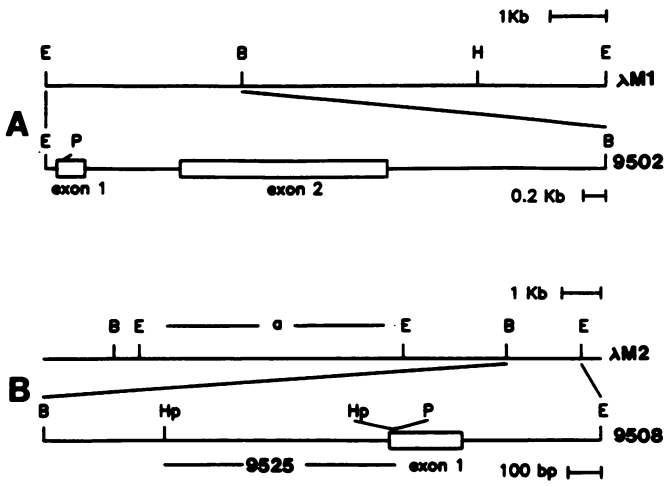

Fig. 1. Restriction maps of the two maize DNA fragments isolated from genomic libraries and the subclones that encode maize hsp70. (**A**) λM1 and pMON9502. (**B**) λM2 and pMON9508. B = *Bam*HI, E = *Eco*RI, H = *Hpa*II, P = *Pvu*I, a = unmapped *Eco*RI site. The structures of the two maize *hsp70* genes, as deduced from their nucleotide sequences, are presented.

© IRL Press Limited, Oxford, England

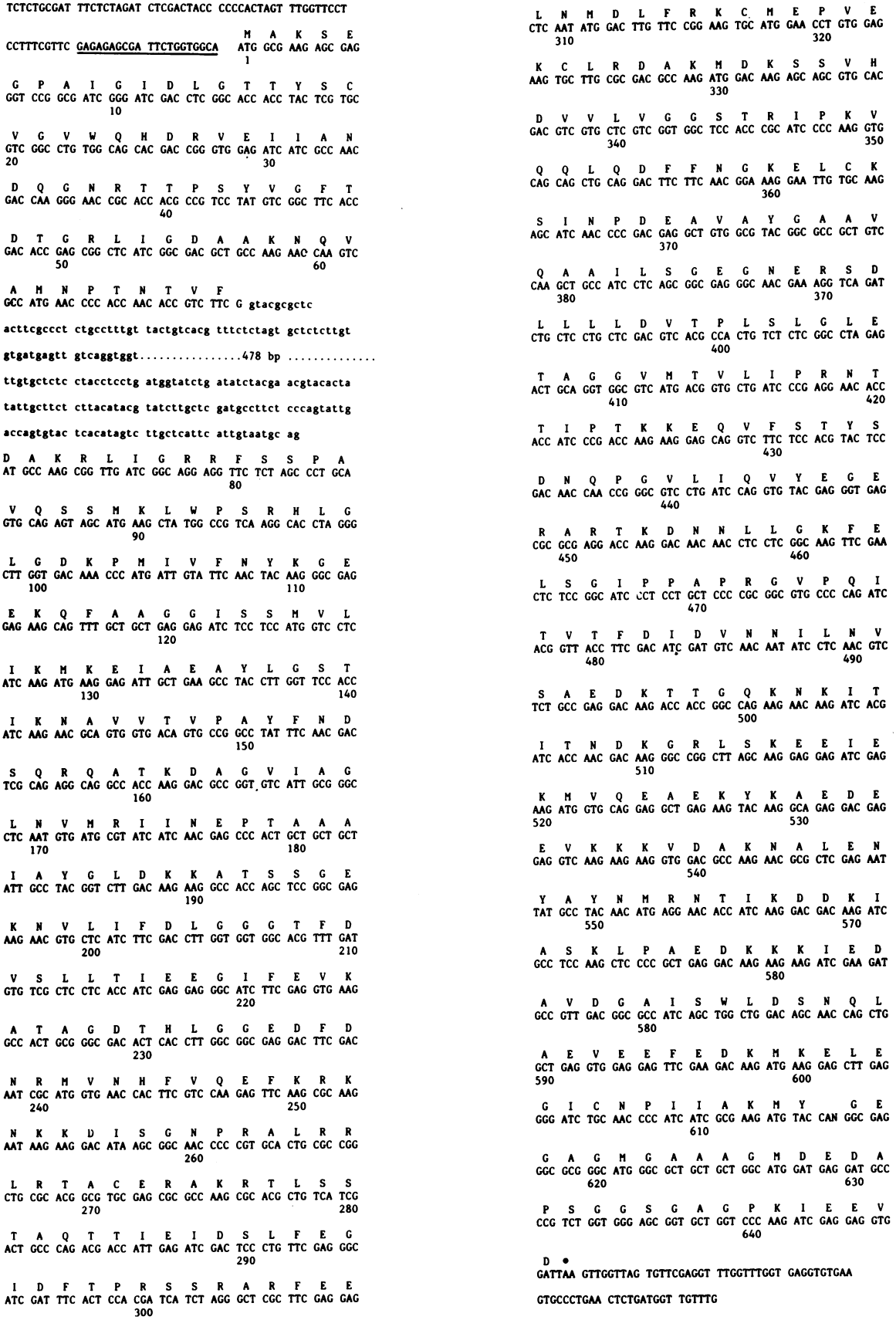

Fig. 2. The complete nucleotide sequence of the maize *hsp70* gene encoded by pMON9502. The sequence spans 81 nucleotides of 5′ nontranslated region, the complete coding sequence of the maize hsp70 polypeptide and 66 nucleotides of 3′ nontranslated sequence. The deduced amino acid residues are denoted above each in the standard one-letter code. The underlined 21-nucleotide-long sequence is complementary to the synthetic probe used in Northern analysis in Figure 5C.

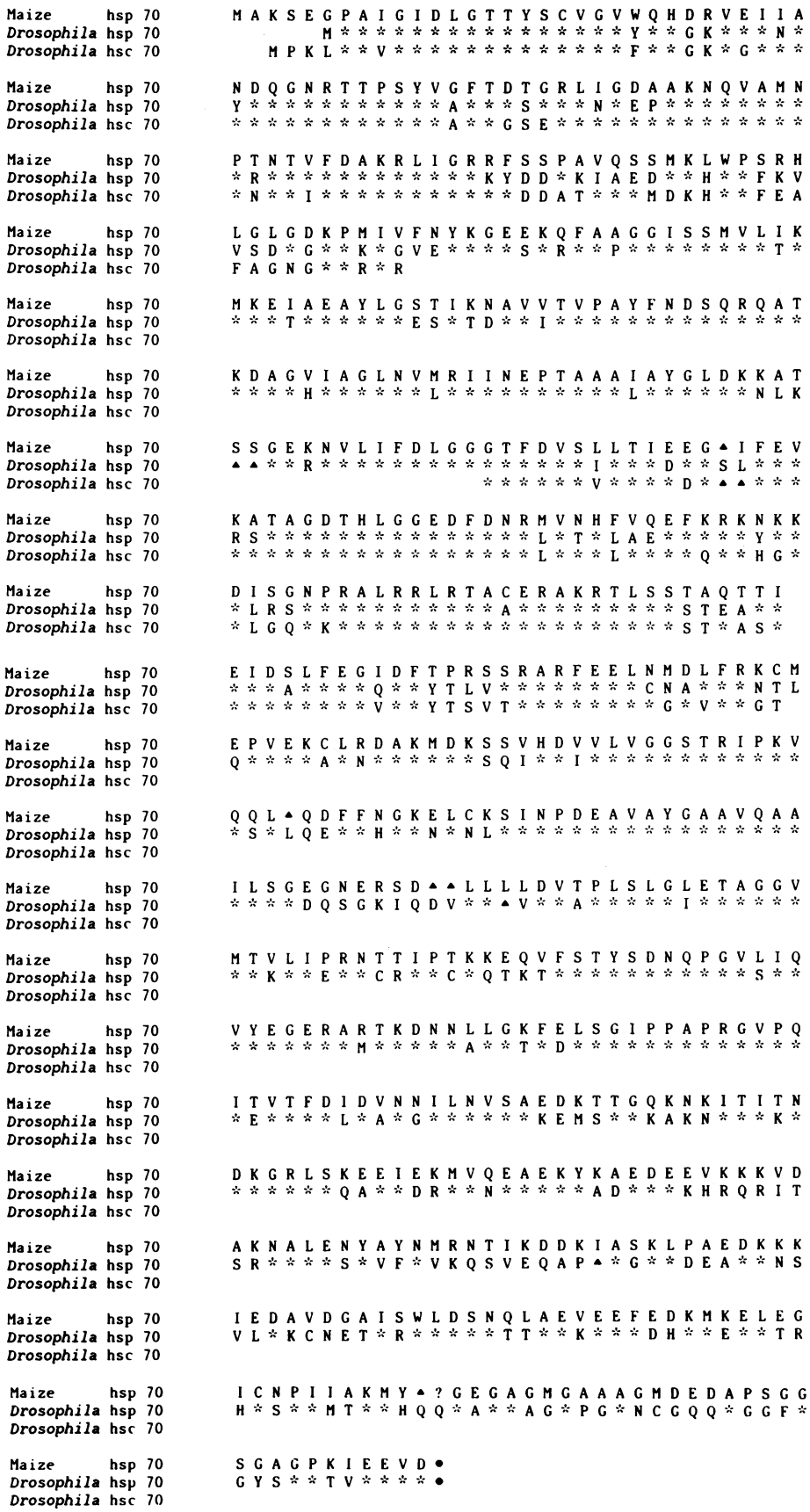

Fig. 3. The comparison of the amino acid sequences of maize hsp70, *Drosophila* hsp70 and *Drosophila* hsc1 polypeptides. Amino acids encoded by *Drosophila hsp70* and *hsc1* genes are shown where differences occur relative to the maize sequence. Only 210 amino acids of the hsc1 polypeptide were available for comparison. Since amino termini of these proteins are heterogeneous, comparison begins with the proline residue at position 7. ▲ denotes the deletion of an amino acid at that position.

terminal heterogeneity has also been observed among *Drosophila* heat-shock-cognate (*hsc70*) genes (Craig *et al.*, 1982), yeast *hsp70* genes (Ingolia *et al.*, 1982) and *Xenopus hsp70* genes (Bienz, 1984). Because of this heterogeneity, analysis of the amino acid sequence homology began with amino acid proline at position 7. As elucidated in Figure 3, the predicted amino acid sequence of maize hsp70 is 68% homologous to *Drosophila* hsp70 and 75% homologous to *Drosophila* hsc1 polypeptide (Ingolia and Craig, 1982). The hsc1/hsp70 comparison is limited to the 210 amino acids of hsc1 sequence that are available. Of the 634 amino acids compared between maize and *Drosophila* hsp70s, the amino terminal 483 amino acids have 75% homology whereas the carboxy terminal 151 amino acids show only 46% homology. Thus, it appears that maize hsp70 has diverged significantly from *Drosophila* hsp70 towards its carboxy terminal end. Bienz (1984) has recently reported that the carboxy terminal quarter of the *Xenopus hsp70* gene is much more diverse than the amino terminal three-quarters. Our observations support her contention that there is much less selective pressure on the function of the carboxy terminal domain or that the carboxy terminal domain is species-specific.

The nucleotide sequence of the λM2 *hsp70* gene subcloned into pMNO9508 (see Figure 1B) was determined and is shown in Figure 4. pMON9508 contains 1.1 kb of the 5′ flanking region and the first exon of an *hsp70* gene. A comparison with the pMON9502 sequence reveals that the homology is restricted to the 210 bp of the entire first exon. The sequence 3′ to this short stretch of homology contains stop codons in all three reading frames, is AT-rich and has a consensus 5′ splice junction sequence. We conclude that the maize *hsp70* gene in pMON9508 also contains an intron in a position identical to that in the *hsp70* gene of pMON9502. Furthermore, we have shown that the *hsp70* gene in pMON9508 is expressed *in vivo* during heat shock in both maize and as part of a hybrid gene in petunia (see Figures 5 and 7) and, therefore, is not a remnant of a pseudogene. These two genes share 88% nucleotide sequence homology and >90% amino acid sequence homology in the first exon.

Expression analysis of the maize hsp70 genes

We have used two leader sequence hybridization probes to examine the expression of the pMON9508 gene. One probe, assumed to be gene specific because it hybridizes to a single band on a Southern blot of maize DNA (see Figure 7), is a 0.7-kb *Hpa*II fragment (pMON9525) subcloned from pMON9508. This probe contains 5′ flanking sequences, the entire 5′ leader and the first seven codons of the coding sequence. The insert from this subclone was used to probe RNA blots containing total and poly(A)$^+$ RNAs isolated from normal (25°C) and heat-shocked (42°C) maize coleoptile tissue (Figure 5A). A major transcript of 2.2 kb and two minor transcripts of 2.6 and 1.0 kb are detected in the RNA from the heat-shocked tissue. All three transcripts are also detected at trace levels in the RNA from normal tissue. While the induction of the 2.2-kb transcript is quite pronounced in response to heat shock, the levels of the 2.6- and 1.0-kb transcripts do not appear to be elevated.

Since there were no convenient restriction sites for making a leader-specific probe for the pMON9502 gene, we synthesized an oligonucleotide homologous to the pMON9502 leader sequences. In order to have confidence in the fidelity of an oligonucleotide probe, we also synthesized an oligonucleotide to the leader sequence of pMON9508 and compared the hybridization pattern of the pMON9508 oligonucleotide probe to that of the

```
-163
GTCGAAACAC GGTTTCCTCT AACTCATTCG GTTCAACCAA CACCCTCCAC

-113
TCCTCCAGAG CCTTCCAGAA CCCCAATCTA ACGGCCAAGG TCGCCCCGTG

-63
CCCGAATCTT CTGGACGCGC CATCTCCTCA ATAAAACCTC CTCGGCTCCT

-13          *
CCAGTGTCCC TCGTCTCTCG CCTGAGAAAA AAAATCCACG AACCAATTTC

+63
TCAGCAACCA GCAGCACGAC CTGTGAGGGT TCGAAGGAAG TAGCAGTGTT

+113                    M   A   K   G   E   G   P   A
TTTTGTTCCT AGAGGAAGAG ATG GCA AAG GGG GAG GGG CCG GCG
                        1
 I   G   I   D   L   E   T   T   Y   S   C   V   G   V
ATC GGG ATC GAC CTN GAA ACA ACG TAC TCG TGT GTC GGC GTG
     10                                          20
 W   A   H   D   R   V   E   I   I   A   N   D       G
TGG CAG CAC GAC CGC GTC GAG ATC ATC GCC AAC GAC CAN GGG
                            30
 N   R   T   T   P   S   Y   V   A   F   T   D   T   G
AAC CGC ACC ACG CCG TCC TAC GTT GCC TTC ACC GAC ACC GAG
             40                                      50
 R   L   I   G   D   A   A   K   N       V   A   M   N
CGG CTC ATC GGC GAT GCC GCC AAG AAC NAG GTC GCC ATG AAC
                                60
 P   T   N   T   V   F
CCC ACC AAC ACC GTA TTT G gtacgtgtca gatgacatcc
                     70

tcttcacttc tgtttccttt catttcactg aacttagaat gcccaattag

cagtctagca ctaaggttgt ttgattccat gctgcgggat tttccactaa

taatcgatga tgcagagttt tacttaatca tttgctgcat agtttcacat

tgatgtcgat gcttcagatg cttgtagatc tatcgaaatt tgtttcagtg

agcgacttct gctctgtttc tatggcaaca tgagcagcac taaacttgga

gatctgattt gttcagtgtg agcagtagct gcttgtttat gttctagatt

tgagtaggat cggataggat taacgattag gtggatgcga gctgatttgt

ggaattc
```

Fig. 4. Nucleotide sequence of the maize hsp70 encoded by pMON9508. The sequence spans 270 nucleotides of the 5′ flanking sequence, exon 1 and part of the intron. The deduced amino acid residues are denoted above each codon in the standard one-letter code. The underlined 21-nucleotide-long sequence is complementary to the synthetic probe used in Northern analysis in Figure 5B. The presumed TATA box is overlined. The heat-shock promoter consensus elements are boxed and the start of transcription is marked with an asterisk. Intron sequence is in lower case.

pMON9508 nick translated probe described above. This comparison can be seen in Figure 5A and B. The results of the experiments in which the 25 and 42°C maize RNAs are probed with pMON9502 leader-specific oligonucleotide probe are shown in Figure 5C. Both oligonucleotide probes, which share no homology, hybridized to a 2.2-kb mRNA in the total as well as the poly(A)$^+$ RNAs from heat-shocked tissue. The levels of the

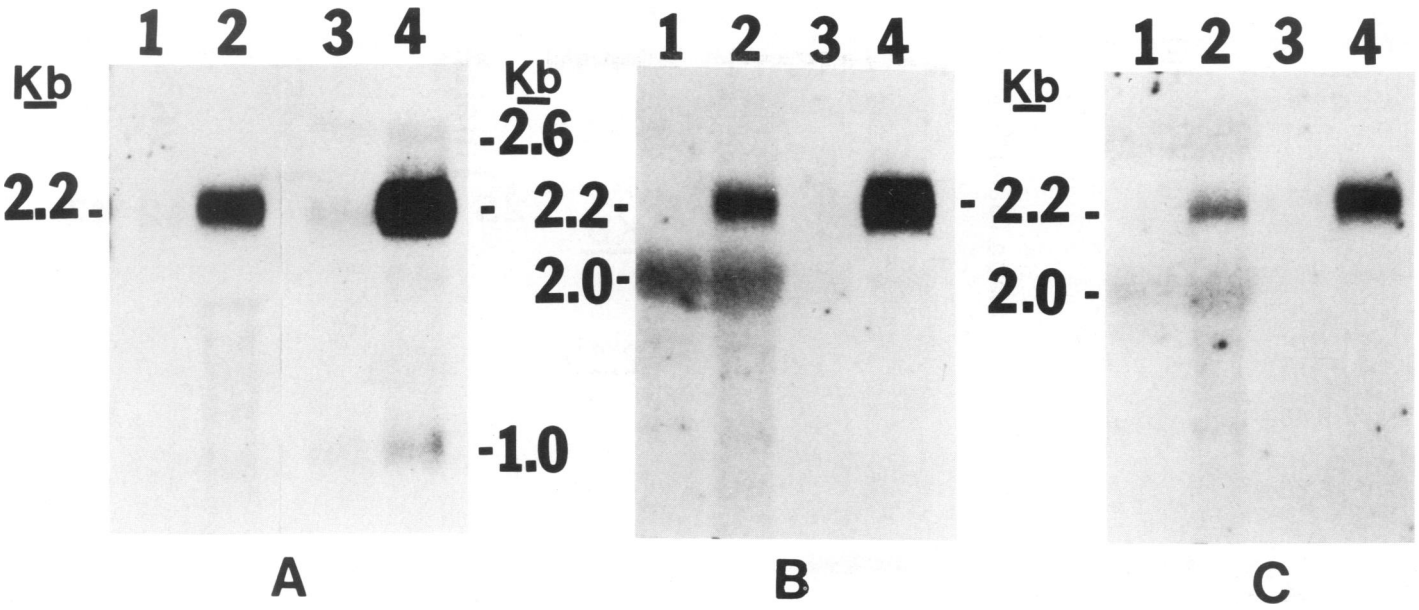

Fig. 5. RNA blot analysis of total and poly(A)$^+$ RNAs from normal (25°C) and heat-shocked (42°C) maize shoot tissue. **Lane 1**: 20 μg of 25°C total RNA; **lane 2**: 20 μg of 42°C total RNA; **lane 3**: 2 μg of 25°C poly(A)$^+$ RNA; **lane 4**: 2 μg of 42°C poly(A)$^+$ RNA. (**A**) Hybridization with pMON9525 insert (see Figure 1B). (**B**) Hybridization with pMON9508 leader-specific synthetic oligonucleotide probe (see Figure 4). (**C**) Hybridization with pMON9502 leader-specific synthetic oligonucleotide probe (see Figure 2). These two synthetic oligonucleotide probes were labeled with ^{32}P using T4 polynucleotide kinase.

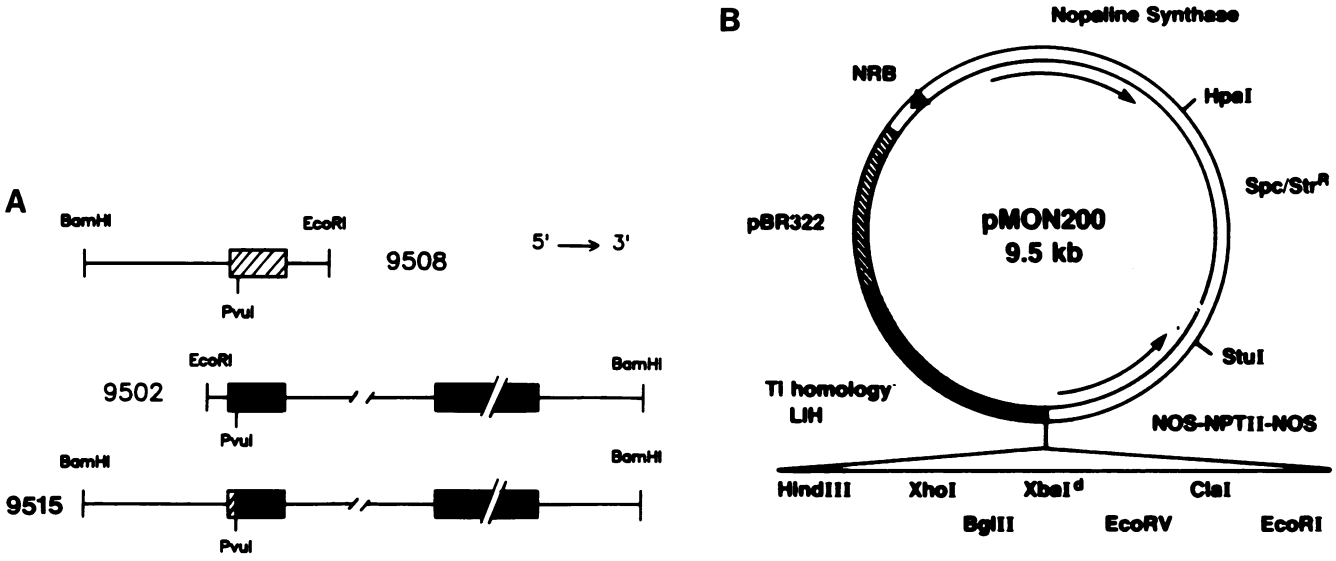

Fig. 6. Construction of a maize hybrid gene for transfer into petunia. (**A**) Both pMON9508 and pMON9502 contain a unique *Pvu*I site in the first exon. The plasmid pMON9515 was constructed by ligating the two clones at this conserved *Pvu*I site and subsequently into the *Bam*HI site of pUC9 (pMON9515). (**B**) The *EcoRI*−*Hin*dIII insert of pMON9515 was cloned into the plant transformation vector, pMON200 creating pMON9516.

other transcripts (2.6 and 1.0 kb) were too low to be detected with the synthetic oligomers. A minor 2.0-kb band observed in the lanes containing total RNA is due to nonspecific binding of the oligonucleotide to 18S ribosomal RNA. The above results indicate that the genes we have isolated are expressed at very low levels at the normal temperature of 25°C and that their expression is induced by heat shock.

Expression of the maize hsp70 gene in transgenic petunia

The construction of a hybrid gene from the two maize *hsp70* clones is outlined in Figure 6. This hybrid gene was transferred into petunia cells and a transgenic plant was generated from the transformed tissue. Based on the genomic blot shown in Figure 7, we estimate that the transgenic petunia plant, 3457, contains 1−2 copies of the hybrid maize *hsp70* gene. The control lane (VR) demonstrates that the probe, pMON9525, does not hybridize to any petunia DNA sequences.

Poly(A)$^+$ RNA was isolated from heat-shocked and normal leaves of the transgenic petunia and analyzed for the presence of a transcript homologous to the maize pMON9525 probe. Figure 8 (lanes 3 and 4) demonstrates that the hybrid maize *hsp70* gene is thermally induced in the transgenic petunia plant. Control lanes indicate that the probe does not hybridize to transcripts from heat-shocked wild type or vector-only transformed petunia. This blot was rehybridized with a petunia *hsp70* cDNA clone (J.Winter, in preparation) to verify that the petunia leaves had been appropriately heat-shocked, and with a petunia extension cDNA clone (C.Hironaka, in preparation) to verify that all of the 25°C lanes contained RNA (data not shown).

Analysis of mRNA start sites in maize and transgenic petunia

The primer extension products shown in Figure 9 demonstrate

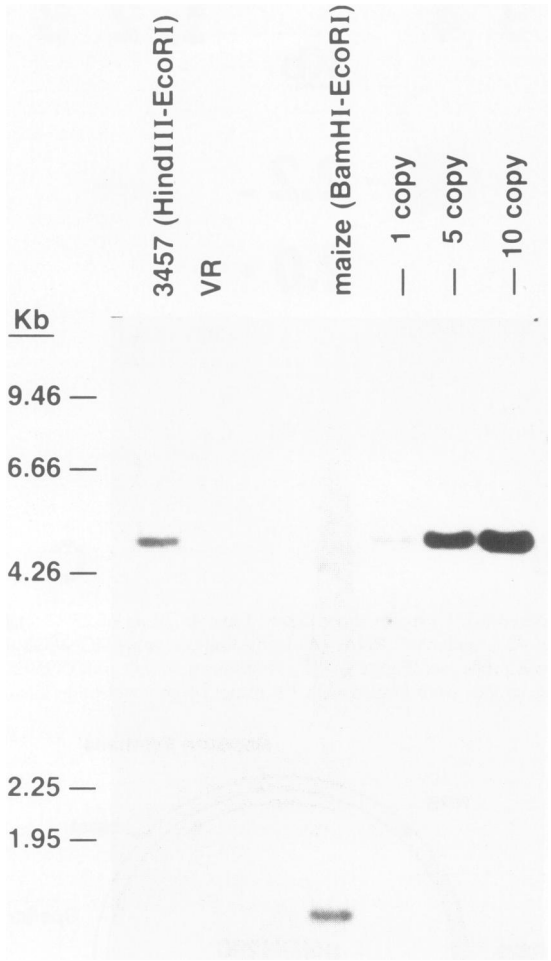

Fig. 7. Genomic blot analysis of leaf DNA from transgenic petunia plant, 3457, containing the maize hybrid *hsp70* gene (pMON9516). VR is the *Hin*dIII−*Eco*RI digest of the leaf DNA from untransformed petunia. Five micrograms of petunia DNA was run in each lane. The maize lane contains 10 μg of Missouri 17 maize DNA. The copy reconstructions were done using the pMON9515 insert. The sizes of the mol. wt. standards are shown in kb.

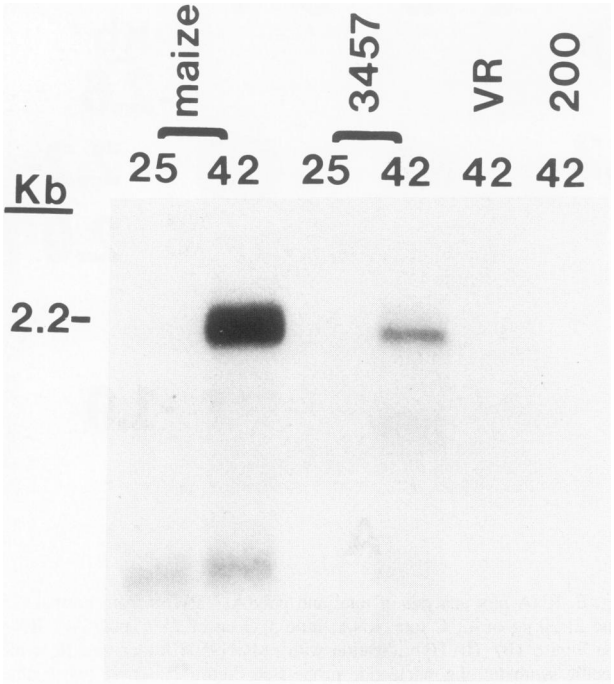

Fig. 8. RNA blot analysis of poly(A)$^+$ RNAs from normal (25°C) and heat-shocked (42°C) tissue from maize shoots and petunia leaves. The VR and 200 lanes contain 42°C poly(A)$^+$ RNA from the leaves of untransformed and vector-only petunia plants, respectively. Two micrograms of poly(A)$^+$ RNA were used in all lanes. The insert in pMON9525 was used as a hybridization probe.

that the major transcription start site is the same for the endogenous pMON9508 gene in maize, and for the hybrid gene in the transgenic petunia plant. The size of these extension products indicates that the 5′ nontranslated leader is 107−109 bp in length and that transcription initiates 34 bp downstream from a TATA-like region (AATAAA). Two sequence elements that match the consensus heat shock element in 8/10 positions are found 29 and 75 nucleotides upstream of the TATA box (Figure 4). We conclude that maize *hsp70* gene is expressed from its own promoter in transgenic petunia.

Discussion

Hsp70 genes are conserved even among distantly related organisms. The predicted amino acid sequence of the maize hsp70 is 68% homologous to *Drosophila* hsp70 (Ingolia *et al.*, 1980) and 75% homologous to both the *Xenopus* (Beinz, 1984) and human hsp70 (Hunt and Morimoto, 1985). Even more striking is the observation that the dnaK protein of *Escherichia coli* (Bardwell and Craig, 1984) and the maize hsp70 are 47% identical. Likewise, the 5′ flanking sequences of the hsp70 genes characterized to date contain a highly conserved sequence referred to as the 'heat shock element' (Pelham, 1982). Previously, Pelham and Bienz (1982) demonstrated that a synthetic copy of this element was sufficient to confer heat inducibility upon the thymidine kinase gene. Some heat-shock genes (Schöffl *et al.*, 1984), including the maize hsp70 gene, have multiple copies of sequences similar to this element upstream from the TATA box.

The maize *hsp70* genes we examined have coding and regulatory sequences that are similar to other *hsp70* genes. The structure of the maize *hsp70* genes, however, differs from all others described to date. The maize genes are unique in that they each contain an intron. The location of this intron is interesting from an evolutionary perspective since the *hsc1* of *Drosophila* also has an intron in the same position. The *hsc1* gene is, however, constitutively expressed and not thermally induced. We have demonstrated that the two maize *hsp70* genes described in this paper are thermally inducible. At normal temperatures, these genes are transcribed at a low level. At 42°C, the transcript level increases 40−60-fold (data not shown).

We have transferred a hybrid maize *hsp70* gene to petunia. This hybrid gene contains 1.1 kb of 5′ flanking region, the entire coding region with an intron and 1.0 kb of the 3′ flanking region. We have demonstrated that 1.1 kb of the 5′ flanking sequence is sufficient for thermal inducibility. The transcript made in petunia has the same 5′ end and co-migrates with that of maize. Previously, other investigators have transferred the soybean *hsp17.5* gene to sunflower (Schöffl and Baumann, 1985; Gurley *et al.*, in press) and demonstrated its induction in tumor tissue. Spena *et al.* (1985) have demonstrated that the hsp70 promoter of *Drosophila* functions in tobacco tumors. These findings, in addition to our observation that a monocotyledonous *hsp70* gene appears to function normally in a regenerated dicotyledonous plant, indicate that the entire heat shock induction system may be highly conserved among diverse organisms.

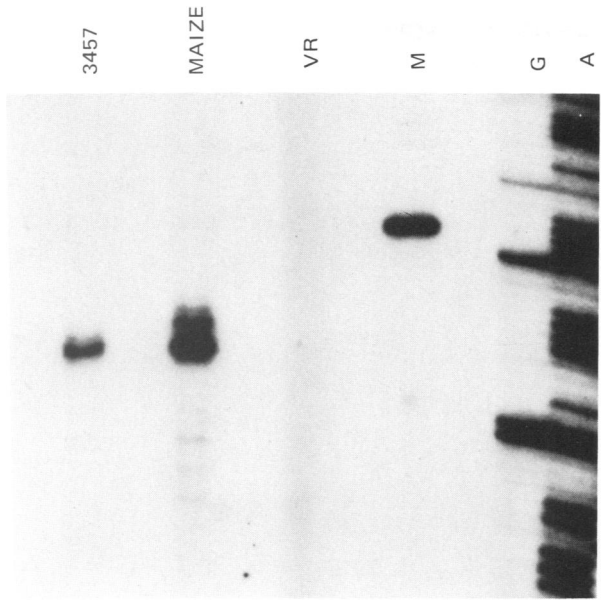

Fig. 9. Primer extension analysis of heat-shock mRNA (42°C) from the transgenic petunia plant. The primer used was the same as in Figure 6B. One microgram of poly(A)$^+$ RNA was used in each extension experiment. 3457 is the transgenic petunia plant containing the maize hybrid *hsp70* gene. The maize line used was Missouri 17. VR is the wild-type untransformed petunia. M is the 188-bp *Hae*III fragment of ϕX174 DNA. G and A are lanes from a known sequence ladder (T and C lanes not shown).

Materials and methods

Construction and screening of maize genomic libraries

DNA was isolated from leaves of young maize plants (inbred line Missouri 17) by the procedure of Mascia *et al.*, 1984). A recombinant phage library was prepared from a partial *Eco*RI digest of maize DNA following the procedures of Maniatis *et al.* (1982) using λgtWES.λB as the vector.

A second library was constructed in the vector λMG14, using 10–20-kb fragments of maize (hybrid line 3780A) DNA partially digested with *Mbo*I, according to the procedures described by Maniatis *et al.* (1982). λMG14, obtained from Dr M.Olson of the Washington University School of Medicine, St. Louis, is a *Bam*HI vector which accommodates ~ 8.5–21.0 kb of foreign DNA. The central stuffer fragment contains multiple *Eco*RI sites which facilitate the isolation of vector acms.

The 3780A DNA library was screened (Nagao *et al.*, 1981) with a *Drosophila hsp70* clone, 232.1 (Livak *et al.*, 1978), which contains 0.93 kb of the hsp70 coding sequence. This resulted in the isolation of a phage, λM1, containing a 9.6-kb fragment of maize DNA. The Missouri 17 library was screened (Nagao *et al.*, 1981) using the 4.0-kb *Bam*HI-*Eco*RI subfragment (see Figure 1A) of the phage λM1 as a probe. This resulted in the isolation of phage λM2 containing 14 kb of maize DNA.

Subclones

A restriction map for the 9.6-kb λM1 insert is shown in Figure 1A. A 4.0-kb *Bam*HI-*Eco*RI fragment homologous to the *Drosophila* hsp70 probe was subcloned into pUC9 and named pMON9502.

A restriction map for the 14-kb λM2 insert is shown in Figure 1B. A 1.5-kb *Bam*HI-*Eco*RI fragment homologous to the λM1 insert was subcloned into pUC9 and named pMON9508.

RNA and DNA isolation

For RNA isolation, maize seeds (Pioneer hybrid line 3780A) were germinated in moist vermiculite in the dark at 28–30°C. The shoot tissue of 5- to 6-day-old maize seedlings was sectioned into 1-cm pieces and incubated in 1% sucrose, 1 mM potassium phosphate, pH 6.0 and 50 μg/ml chloramphenicol for 2 h at 25°C (normal) and 42°C (heat shock) prior to RNA isolation. For petunia RNA isolation, young leaves (1–2 cm in length) from petunia plants were incubated as described above. Total RNA was isolated from the maize and petunia tissue immediately after incubation. The plant tissue was frozen in liquid nitrogen, homogenized in extraction buffer (1% tris-isopropylnaphthalene sulfonic acid, 6% *p*-aminosalicylic acid, 100 mM Tris–HCl, pH 7.6, 50 mM EGTA, pH 7.5, 100 mM NaCl, 1% SDS and 50 mM β-mercaptoethanol) and then extracted with phenol/chloroform/isoamyl alcohol (50:48:2 by vol.) twice. The nucleic acids in the aqueous phase were precipitated with ethanol. The precipitate was dissolved in water and the RNA was precipitated with lithium chloride added to a final concentration of 2 M. The RNA pellet was resuspended in water and ethanol-precipitated. Poly(A)$^+$ RNA was selected by passage over oligo-dT cellulose columns (Bantle *et al.*, 1976). Petunia DNA was isolated by the method described by Shure *et al.*, (1983).

Nucleic acid analysis

For DNA blots, DNA was digested to completion with appropriate restriction enzymes, fractionated on a 0.8% agarose gel and transferred to nitrocellulose. Prehybridization and hybridization were carried out in 30% (low stringency) or 50% formamide (high stringency) containing 5 × SSC, 5 × Denhardt's, 0.1% SDS and 100 μg/ml tRNA at 42°C. Heat denatured nick-translated probe was added in the hybridization buffer at 1.0 × 10^6 c.p.m./ml. The wash conditions were the same as described by Scheller *et al.* (1982) except that the final wash was carried out in 3 × SSC at 50°C (low stringency) or in 1 × SSC at 50°C (high stringency).

For RNA blots, total or poly(A)$^+$ RNA was denatured and resolved on formaldehyde agarose gels, followed by transfer to nitrocellulose. High stringency conditions were used on blots hybridized with nick-translated probes, and, the final wash was carried out in 0.3 × SSC at 65°C. Prehybridization and hybridization with synthetic oligonucleotides was carried out in 6 × SSC, 10 × Denhardt's, 200 μg/ml tRNA at T_m 10°C. The T_m was determined by the formula: 4°C(G+C) + 2°C(A+T) (Suggs *et al.*, 1981). The final wash of these blots was carried out in 6 × SSC at T_m 5°C.

The 5′ end of the maize hsp70 mRNA was mapped in both maize and the transformed petunia according to the primer extension protocol described by Shah *et al.* (1986).

Construction of a hybrid hsp70 gene and plant transformation

Plasmids pMON9502 and pMON9508 were digested with *Pvu*I and *Bam*HI and the inserts (4.0 and 1.1 kb in length, respectively) purified. The inserts were ligated, redigested with *Bam*HI (to obtain the hybrid *hsp70* gene on a linear 5.0-kb *Bam*HI fragment) and cloned into the *Bam*HI site of pUC9 creating pMON9515. This hybrid *hsp70* gene (see Figure 6) was then subcloned as an *Eco*RI–*Hin*dIII fragment into pMON200 (Fraley *et al.*, 1985). The final construct, pMON9516, was mated into *Agrobacterium tumefaciens* and then transferred into the petunia genome via leaf disc transformation (Horsch *et al.*, 1985). The petunia line used was an F1 hybrid of Violet 23 × Red 51.

Acknowledgements

We thank Drs R.Morimoto and M.Meselson for sending us the *Drosophila hsp70* clone, 232.1. We are also grateful to Patsy Guenter and Vicki Grant for typing this manuscript.

References

Bantle,J.A., Maxwell,I.H. and Hahn,W.E. (1976) *Anal. Biochem.*, **72**, 413–427.
Bardwell,J. and Craig,E. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 848–852.
Bienz,M. (1984) *EMBO J.*, **3**, 2477–2483.
Craig,E.A. (1985) *CRC Crit. Rev. Biochem.*, **18**, 239–280.
Craig,E., Ingolia,T., Slater,M., Manseau,L. and Bardwell,J. (1982). In Schlesinger,M., Ashburner,M. and Tissieres,A. (eds), *Heat Shock, From Bacteria to Man*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 11–18.
Fraley,R., Rogers,S., Horsch,R., Eichholtz,D., Flick,J., Fink,C., Hoffmann,H. and Sanders,P. (1985) *Bio/Technology*, **3**, 629–635.
Gurley,W.B., Czarnecka,E., Nagao,R.T. and Key,J.L. (1986) *Mol. Cell. Biol.*, **6**, 559–565.
Horsch,R.B., Fry,J.E., Hoffmann,N.L., Eichholtz,D., Rogers,S. and Fraley,R. (1985) *Science*, **227**, 1229–1231.
Hunt,C. and Morimoto,R. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 6455–6459.
Ingolia,T. and Craig,E. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 525–529.
Ingolia,T., Craig,E. and McCarthy,B. (1980) *Cell*, **21**, 669–679.
Ingolia,T., Slater,M. and Craig,E. (1982) *Mol. Cell. Biol.*, **2**, 1388–1398.
Kimpel,J.A. and Key,J.L. (1985) *Trends Biochem. Sci.*, **10**, 353–357.
Kloopstech,K., Meyer,G. and Ohad,I. (1985) *EMBO J.*, **4**, 1901–1909.
Lin,C.Y., Roberts,J.K. and Key,J.L. (1984) *Plant Physiol.*, **84**, 152–160.
Livak,J.K., Freund,R., Schweber,M., Wensink,P.C. and Meselson,M. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 5613–5717.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 269–294.
Mascia,P.N., Rubenskin,I., Phillips,P.L., Wang,A.S. and Xiang,L.Z. (1984) *Gene*, **15**, 7–20.
Nagao,R., Shah,D., Eckenrode,V. and Meagher,R. (1981) *DNA*, **1**, 1–9.

Pelham,H.R.B. (1982) *Cell*, **30**, 517–528.
Pelham,H.R.B. and Bienz,M. (1982) *EMBO J.*, **1**, 1473–1477.
Scheller,R.H., Jackson,J.F., McAlister,L.B., Schwartz,J.H., Kandel,E.R. and Axel,R. (1982) *Cell*, **28**, 701–719.
Schöffl,F. and Baumann,G. (1985) *EMBO J.*, **4**, 1119–124.
Schöffl,F., Raschke,E. and Nagao,R. (1984) *EMBO J.*, **3**, 2491–2497.
Shah,D.M., Rochester,D.E., Krivi,G.G., Hironaka,C.M., Mozer,T.M., Fraley,R.T. and Tiemeier,D.C. (1985) In Key,J.L. and Kosuge,T. (eds), *Cellular and Molecular Biology of Plant Stress*. Alan R.Liss, New York, pp. 181–200.
Shah,D.M., Hironaka,C.M., Wiegand,R.C., Harding,E.I., Krivi,G.G. and Tiemeier,D.C. (1986) *Plant Mol. Biol.*, in press.
Shure,M., Federoff,N. and Wesser,S. (1983) *Cell*, **35**, 225–233.
Spena,A., Hain,R., Ziervogel,U., Saedler,H. and Schell,J. (1985) *EMBO J.*, **4**, 2239–2743.
Suggs,S., Hirose,T., Miyake,T., Kawasima,E., Johnson,M., Itakura,K. and Wallace,B. (1981) In Brown,D. and Fox,D. (eds), *ICN-UCLA Symposium on Developmental Biology Using Purified Genes*. Academic Press, New York, pp. 683–693.
Vierling,E., Mishkind,M.L., Schmidt,G.W. and Key,J.L. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 361–365.

Received on 13 September 1985; revised on 15 January 1986