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Isolation and Characterization of a Small Heat Shock Protein Gene from Maize

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ABSTRACT

A maize (*Zea mays* L.) genomic clone (Zmempr 9′) was isolated on the basis of its homology to a meiotically expressed *Lilium* sequence. Radiolabeled probe made from the maize genomic clone detected complementary RNA at high fidelity. Furthermore, it hybridized to RNA isolated from staged (an interval that is coincident with meiotic prophase) maize tassel spikelets. Complimentary RNA was strongly (at least 50-fold) induced during heat shock of maize somatic tissue and appeared as a single size class in Northern blot hybridizations. Sequencing of the complete coding region of Zmempr 9′ confirmed the homology of the inferred amino acid sequence to other small heat shock proteins. Consensus sequences found in the flanking regions corresponded to the usual signals for initiation of RNA transcription, polyadenylate addition, and the induction of heat shock genes. The latter sequences conferred heat shock-specific transient expression in electroporated protoplasts when cloned into promoterless reporter gene plasmid constructs. Hybrid-selected translations revealed specific translation products ranging from 15 to 18 kilodaltons, providing evidence that this gene is a member of a related multigene family. We therefore conclude that this maize genomic DNA clone, recovered through its homology to clones for meiotic transcripts in lily, represents a genuine maize small heat shock protein gene.

Imposition of a heat shock initiates an alteration of gene expression in eukaryotic cells ranging from yeast, to higher plants, to vertebrates (see ref. 20 for review). Many of the genes encoding the HSPs[1] from a number of diverse organisms have been cloned and sequenced (see refs. 20 and 23 for review). A set of heat-induced proteins is observed when protein extracts from heat-shocked maize seedlings are examined by SDS gel electrophoresis (9). The low molecular weight HSPs (27, 26, 23, and 21 kD in *Drosophila*, 15–21 kD in plants) are encoded by multigene families (20, 23); however, their function(s) remain unknown. There are two reports of the developmental expression of some of the small HSPs coincident with meiosis. Zimmerman *et al.* (35) observed that two of the four small HSPs in *Drosophila* were specifically induced in the egg chamber of the ovary during the meiotic period of oogenesis and accumulated in the oocyte. In *Saccharomyces*, Kurtz *et al.* (19) found that the single small HSP was also specifically induced during sporulation and meiosis. Although it may be enticing to suggest the small HSPs may serve some function during meiosis, there are no data at present that support this premise.

Recently, it was determined that the lily EMPR clones, a set of related cDNA clones representing transcripts of a repeated gene family specifically expressed during meiosis (3), show substantial sequence homology with a cloned small HSP gene from soybean (5). We recovered a maize genomic DNA clone using the coding portions of lily meiotic cDNA clones as probes. Sequencing of a segment showing the strongest cross-reaction to the lily meiotic cDNA clones showed that the inferred amino acid sequence of this region was homologous to small HSPs, as well as to the lily EMPR sequences (5). Here, we report a detailed characterization of this clone and provide evidence that the low molecular weight heat shock or heat shock-related gene(s) are expressed during the meiotic prophase interval of maize spikelets.

MATERIALS AND METHODS

Recovery and Subcloning of Zmempr 9′

A maize (*Zea mays* L.) genomic DNA Charon phage library made from DNA isolated from "black Mexican sweet corn" was probed with fragments containing the most conserved segments of the lily meiotic cDNA clones pLEc6 and pLEc2 (5) at reduced stringency (5× SSPE), 50% formamide, 1× Denhardt's solution, 0.2% SDS; 40 μg/ml *Escherichia coli* DNA; 32°C). The *Eco*RI fragment from the maize genomic clone containing the homology to the conserved internal fragment of the lily meiotic cDNA clones was identified, and subclones of the entire *Eco*RI fragment (pZm9′R-2) and an *Eco*RI/*Pst*I subfragment (pZm9′-8) were placed into pBR322. From the plasmid subclones, 1865 bp of DNA were sequenced in both directions on both strands. Sets of deletion clones were made in the regions where there were no convenient restriction sites.

RNA Isolation and Characterization

Total RNA from 3- to 5-d-old maize seedlings was isolated using the procedure of Sachs *et al.* (30). Selected maize seedlings were incubated at 40°C for 2 h in a solution of Murashige-Skoog salts before RNA isolation. RNA was also

[1] Abbreviations: HSP, heat shock protein; EMPR, expressed meiotic prophase repeat; CAT, chloramphenicol acetyl transferase; GUS, β-glucuronidase; HSE, heat shock element; bp, base pair(s); poly A, polyadenylate.

isolated from staged developing maize spikelets (6). RNA was prepared for 1.5% agarose formaldehyde gel electrophoresis and subsequent Northern blotting using the procedure of Fourney *et al.* (13). The RNA was capillary blotted to nitrocellulose, rinsed in 2× SSPE for 5 min, air dried, and dried *in vacuo* at 80°C for 2 h. Dot blots of RNA isolated from control seedlings (25°C) and seedlings heat shocked (40°C) for 2 h were prepared using the procedure of White and Bancroft (31). Dot blots of staged maize spikelet RNA were prepared as described by Bouchard (5).

Hybridization of pZm9′ Probes to Maize

A 250-bp *Ava*I fragment from pZm9′-8, covering the portion that is most conserved relative to the lily EMPR clones, was used to probe RNA dots and Northern blots of RNA from maize seedlings (25 and 40°C treated). Hybridizations to the maize RNA dots were performed at "high" (5× SSPE, 50% formamide, 0.5% SDS; 45°C), "medium" (5× SSPE, 50% formamide, 0.5% SDS; 32°C), and "low" (5× SSPE, 20% formamide, 0.5% SDS; 45°C) stringency conditions. Maize RNA Northern blots were probed at high stringency only. To examine fidelity of reassociation of the probe, filters were washed in 0.1× SSPE at increasing temperatures and reexposed.

Hybrid Selection *in Vitro* Translation

Total maize RNA was hybridized to base-denatured plasmid DNA immobilized on nitrocellulose squares. After extensive washing, bound RNA was eluted with boiling water. ^{35}S-Methionine-labeled products from RNA translated *in vitro* with a rabbit reticulocyte kit (Bethesda Research Labs) were analyzed by 13.5% SDS-PAGE.

Reporter Gene Assays

A reporter gene plasmid (pZO30) containing the CAT gene (Pharmacia), the poly A addition region from the nopaline synthetase gene, and no eukaryotic promoter was described previously (2). This plasmid and a similar one (pZO70) constructed with the GUS gene (17) were used to functionally assay the promoter region. A plasmid with the 35S promoter from cauliflower mosaic virus, the CAT gene, and no poly A addition region (pZO50) was used to functionally assay the poly A addition region of the isolated small HSP gene. CAT assays were performed using either the [^{14}C]chloramphenicol technique as described by Gorman *et al.* (15) or by the HPLC method described by Young *et al.*(32). GUS assays were done according to the procedure of Jefferson (16). Electroporations of maize black mexican sweet suspension cell and carrot

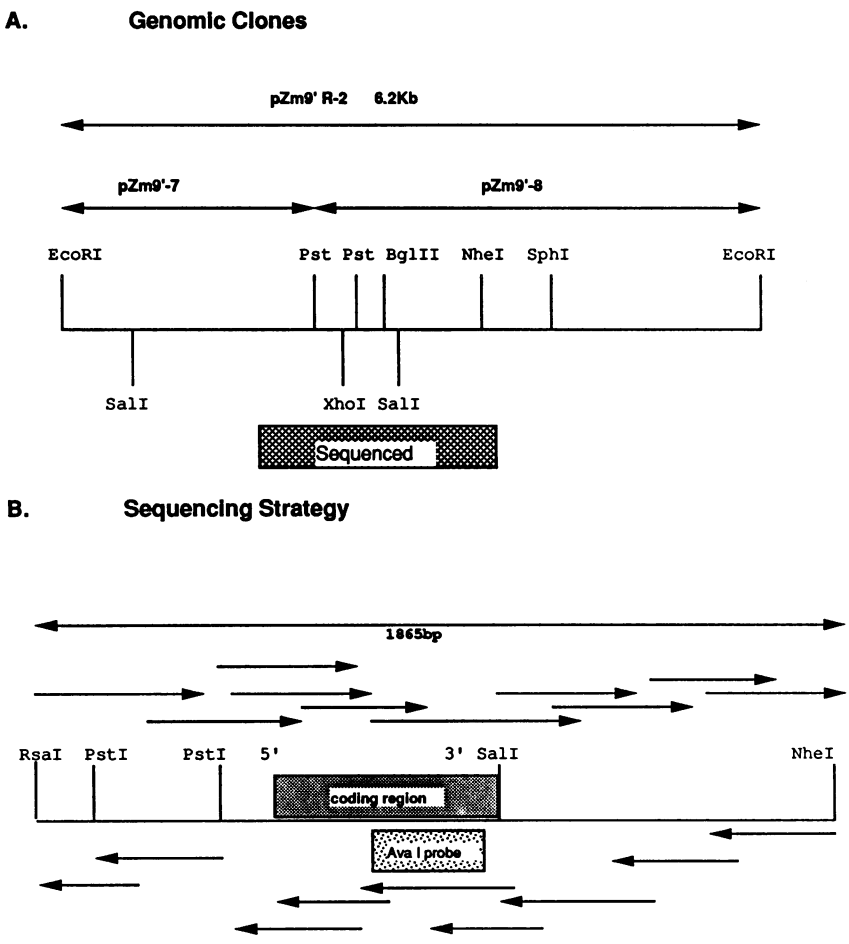

Figure 1. A, Restriction enzyme diagram of genomic clone pZm9′-R2, indicating subclones pZm9′-7 and 8 and the region sequenced. B, Diagram depicting the sequencing strategy, the presumptive coding region, and the *Ava*I restriction fragment used to probe the Northern and RNA dot blots.

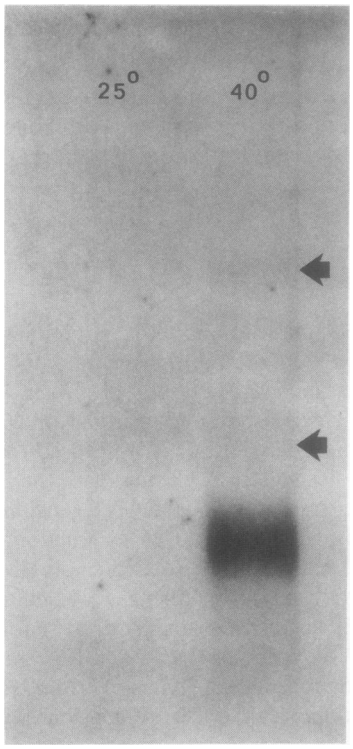

Figure 2. Northern blot of RNA isolated from maize seedlings treated at 25 and 40°C. The probe used (*Aval* fragment of pZm9′-8) encompasses the majority of the open reading frame from the genomic clone Zmempr 9′. Arrows, Positions of the ribosomal RNA subunits.

suspension cell protoplasts were carried out as described by Fromm *et al.* (14).

RESULTS

RNA Analysis

Dots of RNA isolated from maize seedlings treated at 25 and 40°C were hybridized with a nick-translated 250-bp *AvaI* fragment from pZm9′-8 (Fig. 1) that contains only presumptive coding regions of the gene. The enhancement of signal was 50-fold (as determined by densitometric scanning) for heat shock seedling RNA as compared with unstressed seedling RNA, under high, medium, or low stringency (data not shown). Successive washes and exposures were performed at 50, 60, and 72°C in 0.1× SSPE without altering the relative signal strengths, indicating that the heat shock RNA includes components with high pairing fidelity to the probe (not shown). Only under the lowest stringency hybridization conditions was a substantial signal (about one-fifth that of the heat shock RNA) seen for unstressed seedling RNA, and this unstressed tissue signal was effectively removed by a wash at moderate stringency (50°C in 0.1× SSPE).

Northern blot analysis showed a band only in the lane containing RNA isolated from heat-stressed seedlings. This band is broad but single, suggestive of a range of different length RNAs (Fig. 2). The approximate molecular size of the RNA species detected is 600 bp, consistent with size expectations for proteins of 15 to 18 kD. Thus, the genomic sequence from the pZm9′ clones is complementary to RNA species of the correct size for small HSP gene messages, and these are induced under heat shock conditions in maize seedling tissue and are undetectable in unstressed seedling tissue. The synchronous development of lily microsporocytes allows the facile isolation of meiotic stage-specific RNA. The pZm9′ fragment probe hybridizes to staged lily microsporocyte RNA much more weakly than lily EMPR probes (data not shown) but shows a similar pattern of peak hybridization (meiotic prophase interval) to RNA isolated from staged maize spikelets (Fig. 3). There is no detectable hybridization to RNA isolated from mature spikelets containing pollen or from mature pollen. A similar pattern of hybridization to spikelet RNA was observed when a gene-specific fragment (3′-untranslated portion of the gene) was used to probe the dot blots (data not shown).

In vitro translations of maize seedling (40°C) heat shock RNA hybrid selected with a subclone of pZm 9′ (containing only presumptive coding sequence) provide evidence that the genomic clone is a member of a multicopy family of genes.

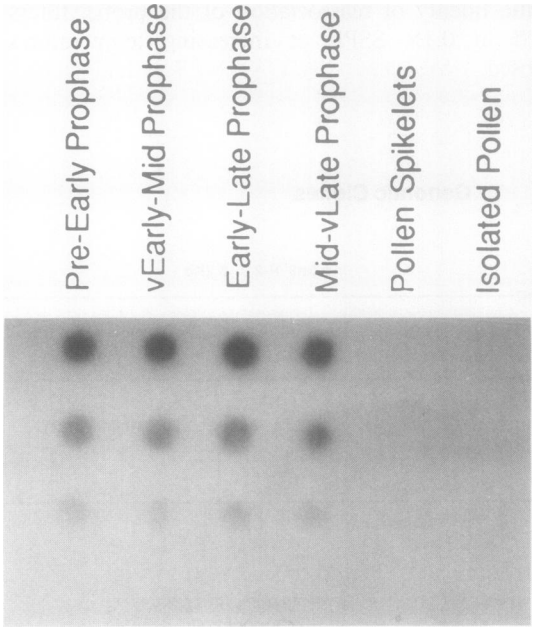

Figure 3. Dot blots of RNA isolated from staged maize spikelets. Each lane is composed for four dots containing 10.0, 5.0, 1.0, and 0.1 μg of total RNA isolated from staged maize spikelets. These have been hybridized to a nick-translated fragment from pZm9′-8 (*Aval* probe) which contains presumptive coding regions of the HSP gene using conditions described in "Materials and Methods" as "high." The relatively crude staging of the maize spikelets was done as described by Bouchard and Walden (6). The pre-Early Prophase material includes maize tissue approaching meiotic prophase and some entering leptotene/zygotene. The vEarly-Mid Prophase material contains RNA from very early prophase and from late zygotene/pachytene stages, whereas the Early-Late Prophase covers the leptotene/zygotene and late pachytene intervals. The Mid-vLate lane contains RNA isolated from maize spikelets representative of late pachytene and diplotene stages. The pollen RNA is isolated from spikelets containing mature predehiscent pollen, and the Isolated Pollen RNA is from pollen shed from mature spikelets.

HSP 18

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     -590       -580       -570       -560       -550       -540       -530
ACTCCCTCCG TCTCAGGATA TAAGGCGTAA CCACTTTTTA TTCTTGTCCC ACAATATAAG GCGTGCTCTC

     -520       -510       -500       -490       -480       -470       -460
TCTATGCATA CGTATATCGA TGCAGTGGTA TAGAGACAAT TAAATGCATT TCTTGGTCTT TGAACCAGAG

     -450       -440       -430       -420       -410       -400       -390
GTGGTTACGC CTTATATCTG GGACGGAGGG AGTAATGCCA AAGCATTACA TATTTTTAAA CAATTATTGC

     -380       -370       -360       -350       -340       -330       -320
AGTATTAACA TTAATAGTAT ATTGTTCTAT CTAAAGAGAG GCCTCATTTT AGTTTTTTTT AAGGGGCCAC

     -310       -300       -290       -280       -270       -260       -250
AAATTTTCCT GGCCTGGCCC TGCCGACGTG GACAGGTACA CAGATGGAAC CAGCAGGAGG CACGTGGCTG

     -240       -230       -220       -210       -200       -190       -180
TTGCCACAAG CTACTGACAT ATGGGCCCAG CAAAACCCCA CTGACTCTGC AGAAAGAGCA CCCAAGGTAC
                                                 Pst I
     -170       -160       -150       -140       -130       -120       -110
ACTGACTCTA TTTTATTCCA GACTCTTCCG GAATCCCAAC AACACAGGGC AGGGACGCCT CCGCCTCGCC

     -100        -90        -80        -70        -60        -50        -40
GCGCCAGGCG GCCCGAGCAC ACTCCACCAC CTTTCAGAAC CTTCCATCGC CTTCCAGAAG CCCATCCCTC

      -30         -20        -10          1          10         20         30
ACCACCTATA TAAACCGCCC CCTCCGGCCT CGTCACTCCC CACACCAAGA ATCACACAAC AGCAGCTCAA

       40         50         60         70         80         90        100
AAAACCCAAC ACACCTCAAC AAGCAGAGCA AAGACCCGGG ATTCGCCGGC GCGGTAGCGA GAGCGAGAGA
                                    Sma I
      110        120        130        140        150        160        170
TGGACGGGAG GATGTTCGGT CTCGAGACCC CCCTGATGGT GGCGCTGCAG CACCTTCTGG ACGTGCCCGA

      180        190        200        210        220        230        240
CGGCGACGCC GGCGCGGGCG GCGACAAGGC GAGTGGTGCC GCCGCGGCGG GCGGCGGGCC CACGCGTACC

      250        260        270        280        290        300        310
TACGTCCGCG ACGCGCGCGC CATGGCGGCC ACCCCGGCCG ACGTCAAGGA GCTCCCGGGC GCGTACGCGT

      320        330        340        350        360        370        380
TCGTGGTGGA CATGCCGGGG CTGGGCACGG GCGACATCAA GGTGCAGGTG GAGGACGAGC GGGTGCTGGT

      390        400        410        420        430        440        450
GGTCAGCGGC GAGCGGCGCC GGGAGGAGCG CGAGGACGCC AAGTACCTGC GCATGGAGCG GCGGATGGGC

      460        470        480        490        500        510        520
AAGTTCATGC GCAAGTTCGT GCTGCCGGAC AACGCCGACA TGGACAAGAT CTCGGCGGTG TGCAGGGACG

      530        540        550        560        570        580        590
GCGTGCTCAC CGTGACCGTC GAGAAGCTGC CCCCGCCCGA GCCCAAGAAG CCCAAGACCA TCGAGGTCAA

      600        610        620        630        640        650        660
GGTCGCCTGA GGGGGGACTA GGTCGACCGA TCGTGGCTCG GAAGTCGGAA GATGTGGGAG CGGTGCCAGT

      670        680        690        700        710        720        730
GAGTTGTGTG GAATGTGGTG ACTGGTGAGG AAGAGGAATA ATCCGTAGTC TGTAGTGGGG TGCTTCTGGT

      740        750        760        770        780        790        800
GTGCGTGTTT GCGTTCCTTC GGTCGCGGTG TCTGTCTACC AATTCCTATC TGATGTATCG ACTTCGGTTT

      810        820        830        840        850        860        870
CGTTTGAACC ATGCCGGATG CTTTCAGTAT GTTCATCTAC CTGTGTCTTT TGTCTAAATA ATCCGTAGTC

      880        890        900        910        920        930        940
TTCATTGACA GAAAGACTAC ACACGTTATC CTGACAAGAC ACGAAATCCC AATAAAGTTT AAATTCAGGC

      950        960        970        980        990       1000       1010
TACTACCAAG AACCCAAAAA AATTATAATC AAAATTTCAA GGGCAGAGTC CGGAAGTGCA AAGCAAAATG

     1020       1030       1040       1050       1060       1070       1080
AACCAATAGT TCAAATTCAG GCTATTACCA CCAAGGATAT AAGTTTTATA AGCAAATTGT TTAAGTGCAG

     1090       1100       1110       1120       1130       1140       1150
AACAAAATGA ACCAACAATT CAAAGGCGAA GGCAGGAGTA ACTTGCCGTA GGATTATAGA ATTTCGATTC

     1160       1170       1180       1190       1200       1210       1220
AATGAATCCA GGAAGTTGCA TTCACTCATG ACTTTCGTCT ACAGCTATGA GAAACAGTAA TTTTATAAGC

     1230       1240       1250       1260
AATGTCTCTC AAAACACATG CAGGCCATCC GTTACCTAAA TGCTAGC
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Figure 4. DNA sequence of the genomic clone for a small heat shock gene of maize. The TATAA box (shadowed letters) is numbered −30, and the presumptive mRNA start site (bold letters) is the position 1. The shorter promoter fragment used in the transient assays is defined by the *Pst*I site at −200 and *Sma*I site at 70 in the 5′-untranslated leader. The longer version of the promoter used in the transient assays contains approximately 1725 bp of additional 5′ upstream sequence. Only 598-bp of upstream sequence is shown here. Most previously described heat shock genes have an upstream consensus sequence (HSE) consisting of CTNGAANNTTCNAG that is responsible for the heat-induced expression. A repeated or overlapping heat shock consensus element (HSE) is in bold letters and underlined at −70 to −50. An additional HSE is found at position −150. The presumptive stop codon is at position 621 and the open reading frame from the first ATG (in bold) to this stop codon should encode a protein of 17.9 kD. There are a least three sequences that closely match the consensus sequence for poly A addition 700, 859, and 924.

The hybridization conditions used result in hybrid selection of mRNAs specifying proteins ranging from 15 to 18 kD (data not shown). No detectable proteins were observed when hybrid selected RNA from seedlings treated at 25°C was translated.

Sequencing

After restriction enzyme mapping of the genomic clone (pZm9′ R-2), shorter restriction fragments and a set of nested deletions were subcloned into pTZ18R and pTZ19R for sequencing (Fig. 1), and 1865 bp were sequenced in both directions using Klenow or T7 DNA polymerase (Fig. 4). Within this region, one opening reading frame was found that showed sequence homology to a soybean heat shock gene previously described (10). Consensus sequences for HSE (24), TATAA box, and poly A addition sites were also found within this region. Plant genes usually have an mRNA start approximately 25 to 35 bp after the TATAA sequences (18). If the first ATG after the presumptive transcription start is utilized for protein initiation, the deduced amino acid sequence of the opening reading frame should yield a protein of 17.9 kD. Downstream from the TGA stop codon three presumptive poly A addition sequences were found.

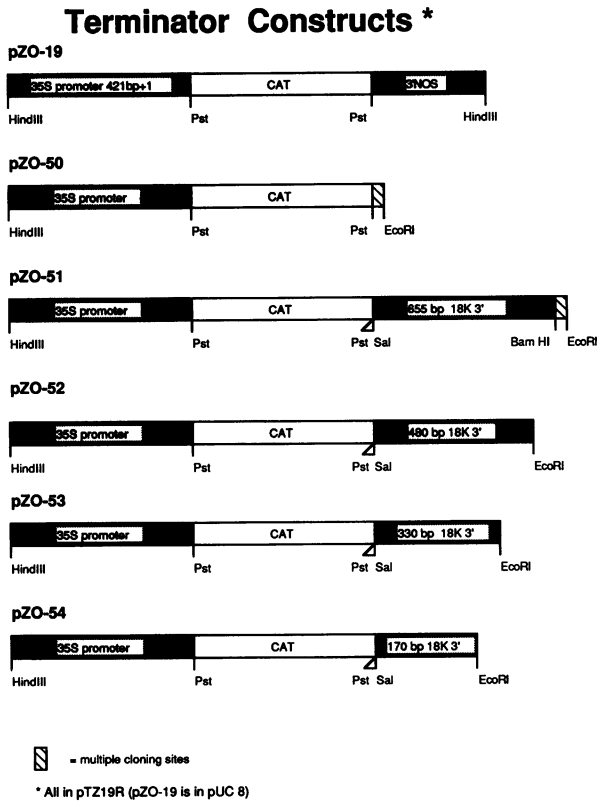

Figure 5. Diagrammatic representation of the plasmid constructs used in the analysis of terminators. All constructs utilize a 421-bp version of the 35S promoter from CaMV, 1 bp of the 35S 5′-untranslated leader, and 18 bp of the CAT 5′-untranslated leader. The terminator from the nopaline synthetase gene serves as a comparative control.

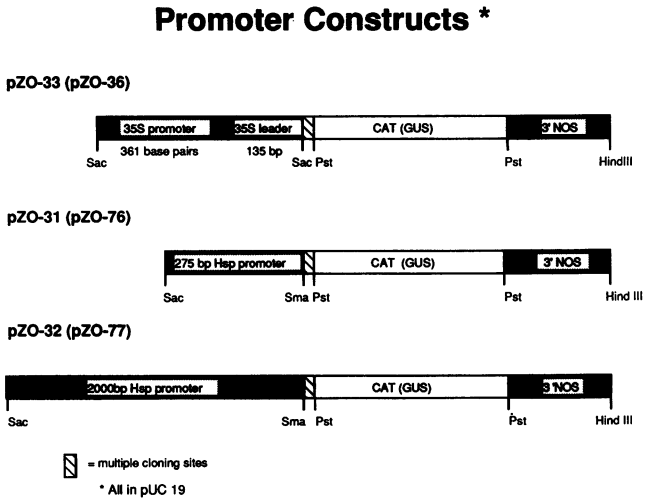

Figure 6. Diagrammatic representation of the plasmid constructs used in the analysis of promoters. All of the plasmids are virtually identical, with only the promoters and 5′-untranslated leaders differing. The only difference between pZO 33 and pZO 19 is that the former contains 135 bp of 5′-untranslated 35S leader *versus* 1 bp in the latter. All constructs include 17 to 18 bp of bacterial 5′-untranslated leader. The heat shock promoter constructs include 70 bp of the 100-bp native heat shock 5′-untranslated leader that is fused to polylinker and the subsequent bacterial gene leader.

Transient Expression Analysis

Because of our interest in using the promoter and poly A addition region to express novel agronomic traits in plants, we analyzed the promoter and poly A addition regions by using transient expression in maize protoplasts. The plasmid constructs used in the analysis are diagrammed in Figures 5 and 6. The transient expression data obtained 24 h after electroporation are shown in Table I. One of the control plasmids, pZO19, has a short 5′-untranslated leader from the 35S promoter and the other (pZO33) contains 135 bp of 35S leader. All CAT constructs include 16 bp of bacterial CAT gene leader. The transient expression levels of pZO33 were consistently threefold higher than pZO19; this is probably due to the inclusion of more of the 35S untranslated leader, because there are no other differences between these two plasmid constructs. With one exception (pZO52), the transient expression levels obtained with the various deletions of

Table I. *Transient Assay Results with the Various Terminator Plasmid Constructs*

The data represent the mean ± SD of two separate experiments; each data point done in triplicate.

Plasmid	% Acetylation	Relative to pZO19
pZO 19	4.30 ± 0.4	1.00
pZO 50	1.05 ± 0.4	0.24
pZO 51	1.55 ± 0.2	0.36
pZO 52	6.00 ± 0.2	1.40
pZO 53	3.70 ± 1.4	0.86
pZO 54	1.90 ± 0.7	0.44
pZO 33	12.9 ± 3.2	3.00

the terminator from the 17.9-kD gene are consistently less than those observed with the nopaline synthetase terminator. Although absolute amounts of acetylated product varied from experiment to experiment, the relative amounts of product generated by the various plasmid constructs remained the same: pZO52 was consistently 1.4 to 1.5 times more active than pZO19. Interestingly, when the same constructs were analyzed in electroporated carrot protoplasts, the longest version (pZO51) of the terminator yielded the greatest amount of acetylated product, and the progressive deletions of the terminator yielded incrementally lower amounts of acetylated product (data not shown).

The plasmid constructs used in the analysis of promoter regions are illustrated in Figure 6. The control plasmid (pZO33) contains the long version of the 35S 5′-untranslated leader region. Transient expression results obtained 24 h after electroporation are shown in Table II. The 17.9-kD heat shock promoter appears to be almost entirely heat inducible in these protoplasts. The heat treatment was carried out at 40°C, which was previously determined to be optimal for induction in the maize protoplasts (7). CAT enzyme activity increased linearly for up to 4 h of heat shock (data not shown). Results obtained with the GUS plasmids were very similar to those with the CAT gene. After 1.5 h of heat shock, either length version of the 17.9-kD heat shock promoter yielded 2 to 2.5 times the amount of reporter gene product compared to the 35S promoter (24 h postelectroporation at 25°C). There appears to be a very low amount of basal (25°C) activity with this HSP promoter in our test system.

DISCUSSION

We have isolated and characterized a heat shock gene that should encode a protein of 17.9 kD. There is an approximately 50-fold increase for mRNAs that cross-hybridize to this gene in heat-stressed maize seedlings. Translation of hybrid-selected RNA demonstrated that there are multiple 15- to 18 kD HSPs in maize. Indeed, this clone has been used by others to isolate several other related genes (B. G. Atkinson, personal communication).

The deduced amino sequence from the maize clone is shown in Figure 7 aligned to other selected small HSP sequences. In general, there appears to be more sequence homology in the carboxy-terminal portion of the low molecular weight HSPs. The sequence "GVLTVTV" found at positions 141 to 147 in the maize protein is conserved in most small HSPs including *Drosophila* (20), but the lily EMPR gene has a 12- to 14-amino acid insertion after this conserved sequence. The maize HSP and the soybean 17.9-kD genes also exhibit homology in the N terminus. The sequence GLE-PL---LQH is shared between these proteins at positions 7 to 18. The maize protein is more closely related (64% or 107 of 167 residues being identical) to the soybean 17.9-kD gene (a class VI protein of the family of small 15- to 18-kD HSPs) than it is to the wheat small HSP, the lily EMPR clone, or the soybean 17.5-kD HSP. Therefore, if the maize protein were to be assigned to a particular class of the 15- to 18-kD family of proteins, it would most appropriately be assigned to class VI.

The flanking regions of this gene have been analyzed for their ability to drive the transient expression of reporter genes in electroporated protoplasts. In our test system the maize promoter is very strongly heat inducible with little detectable basal (25°C) expression. The level of heat-induced expression of the reporter gene is approximately 50-fold more than the non-heat-shocked controls. After 2.5 h of heat stress, the maize heat shock promoter-containing constructs produce approximately 5 times the amount of reporter gene product as do the 35S cauliflower mosiac virus promoter constructs in electroporated corn protoplasts. This is relatively lower than the levels reported by Ainley and Key (1), who reported an 80-fold higher level with the 17.5-kD soybean HSP promoter when compared to their version of the 35S gene promoter-GUS construction in tobacco protoplasts. This apparent discrepancy could be due to the relative strengths of the 35S-containing plasmids in the maize and tobacco protoplasts. The 35S version that we use has been modified for high level of expression in maize protoplasts (12).

In other organisms, there is a preferential translation of heat shock mRNAs during heat stress (20). This is attributed to certain features of the 5′-untranslated leaders of the heat

Table II. *Transient Assay Results with the Various Promoter Constructs in Corn Protoplasts*

The absorbance measurements were taken after 4 h. The various constructs were tested in triplicate and results are means ± SD.

Plasmid	CAT: % Acetylation		GUS: *A*		Heat Treatment Period	Relative to 35S
	− Heat	+ Heat	− Heat	+ Heat		
					h	
pZO 31	<0.05	23.0 ± 0.6			1.5	1.96
pZO 32	<0.05	30.2 ± 1.8			1.5	2.57
pZO 33	10.3 ± 0.5	13.2 ± 1.9			1.5	1.00
pZO 31	1.0 ± 0.9	49.5 ± 0.6			2.5	4.66
pZO 33	10.6 ± 2.0	9.7 ± 1.6			2.5	1.00
pZO 76			0.0015 ±0.001	0.245 ±0.051	1.5	2.45
pZO 77			0.005 ±0.002	0.238 ±0.009	1.5	2.38
pZO 36			0.100 ±0.001	0.095 ±0.005	1.5	1.00

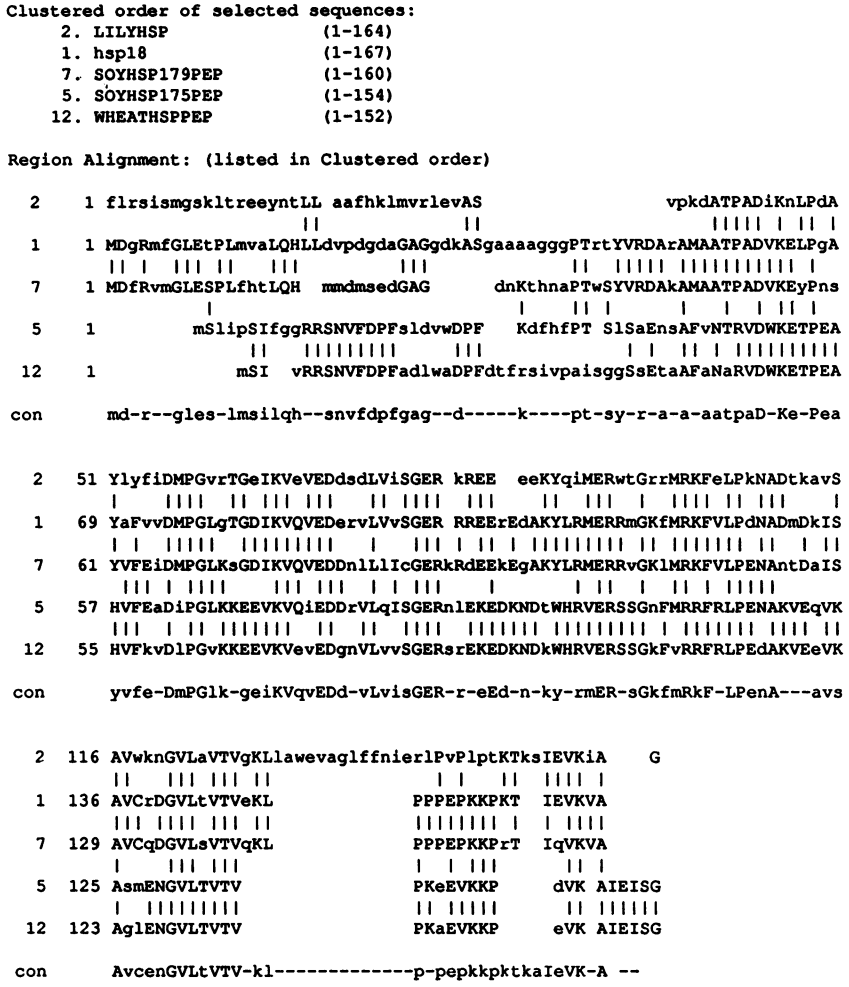

Figure 7. An alignment of selected small HSPs and the derived amino acid consensus sequence. The maize protein sequence is from this study; the soybean 17.9-kD sequence (27), the lily sequence (5), the wheat sequence (21), and the soybean 17.5 kD sequence (10) are the results of other reports.

shock mRNAs. Due the presence of a hybrid (part maize sequence, part bacterial) 5′-untranslated leader in our plasmids, there is the possibility that the heat-induced expression of the reporter genes in our assays is not optimal. We find this to be unlikely for two reasons. First, our plasmid constructs do contain 70 of the 100 bp of the native heat shock gene 5′-untranslated leader (along with the 16 to 17 bp of bacterial reporter gene leader). Computer analysis of this chimeric leader does not show a significant deviation from the native leader in potential secondary structure (or lack thereof). Second, transient expression analysis with constructs containing the full-length and truncated versions of 5′-untranslated leader from an 83-kD maize heat shock gene shows no measurable differences in the levels of reporter gene activity (Casey *et al.*, in preparation). However, we cannot rule out the possibility that our assay system is not sensitive enough to detect small enhancements due to 5′ leader effects.

The various deletions of the terminator region yield varying amounts of reporter gene product in our test system. The 452 bp version of the 18-kD terminator yielded 1.4 to 1.5 times the amount of acetylated product as the nopaline synthetase terminator in maize protoplasts. This result is similar to that observed by Ainley and Key (1), who found equal levels of expression with the nopaline synthetase poly A addition region and the terminator from the soybean 17.5-kD HSP gene. Data obtained from animal systems demonstrate that heat shock mRNA from the 70-kD gene is unstable and the principal component that contributes to the instability is the 3′-untranslated portion of this mRNA(25). To our knowledge there have not been any well-documented reports of mRNA instability attributed to the 3′-untranslated portions of plant heat shock mRNAs. Within the sensitivity limits of our assay system, the maize 17.9-kD heat shock terminator does not appear to be debilitating to transient expression of the reporter gene in maize or carrot protoplasts.

The upstream region of this gene contains similar sequence motifs as found in soybean genes (11). The sequences responsible for heat-induced expression were originally thought to involve a 14-bp motif consisting of TCnGAAnTTCnAG found 14 to 28 bp upstream of the TATAA box (24). There is now a more general definition of the HSE (34) as alternating pentanucleotide patterns of purines and pyrimidines: (nGAAn)(nTTCn)(nGAAn)(nTTCn), which can be effective in directing heat-induced expression at much greater distances from the TATAA box. The maize upstream HSEs closely match this more universal convention of a dimer of nGAAnnTTCn. Our results demonstrate that 275 bp upstream from the mRNA start is sufficient for heat-inducible

transient gene expression in maize protoplasts. Furthermore, the inclusion of additional upstream sequences has little or no effect on the degree of heat inducibility of our reporter genes. It has been demonstrated in *Drosophila* that the developmental control of this class of genes is regulated by more distal sequences (8, 22, 29). It is now feasible to determine whether upstream sequences in this promoter are involved in developmental regulation by making deletion constructs with reporter genes and stably transforming them into maize plants (28).

Studies showing developmentally programmed synthesis of small HSPs in the absence of stress induction in quite different organisms suggest that the HSP relatedness (and possible dual heat shock and meiotic functions) of EMPR or EMPR-like gene products may be of general significance. Zimmerman *et al.* (35) observed that two of four small HSPs in *Drosophila* are specifically induced in the egg chamber of the ovary during the meiotic period of oogenesis and accumulate in the oocyte. In *Saccharomyces*, Kurtz *et al.* (19) found that the single small HSP is also specifically induced during sporulation and meiosis, its transcript eventually accumulating to levels higher than those seen during heat induction, and its product becomes one of the five most abundant cellular proteins (S. Lindquist, personal communication). In this communication we report that maize anthers undergo stage-specific expression of RNAs which hybridize to the small heat shock gene described. This similarity in development as well as stress induction of small HSPs or related genes is striking because, except for the process of meiosis, the gametogenic cell developmental events leading to the yeast ascospore as compared with the fruit fly oocyte or the plant microspore are highly dissimilar. The finding of small HSP or small HSP-like gene transcription correlated with meiosis in organisms from three eukaryotic kingdoms carries the implication that some small HSPs or related proteins may also have a specific function in the meiotic cells of organisms, a function that is part of normal development.

Although striking, the relatedness of the EMPR sequence products in meiocytes to the small HSP gene products induced by heat shock is not of itself illuminating, because the function of the small HSPs in somatic cells remains obscure. Despite the universality in the heat induction of small HSPs, their synthesis does not appear to be necessary for thermotolerance in plants under all situations (4, 33). Moreover, it is known that complete disruption of the single small HSP gene detectable in yeast has no apparent effect on viability or thermotolerance (26). Thus, the function of this class of proteins may be more subtle than has been supposed. Further investigations of the developmentally programmed expression of small HSP genes, or perhaps of their close relatives during meiosis, may help unravel not only their role in meiotic cells but their role in heat-stressed cells as well.

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