

Developmental Expression of Tomato Heat-Shock Cognate Protein 80

Ann J. Koning¹, Ronald Rose², and Luca Comai^{*3}

Calgene Inc., Davis, California 95616; and Department of Botany, University of Washington, Seattle, Washington 98195

ABSTRACT

Heat-shock protein 80 (HSP80) is a major heat-shock protein induced in yeast and animals both by heat shock and by specific developmental events. In plants, a heat-shock-induced HSP80 cDNA has been described, although no information concerning developmental regulation of HSP80 genes is available. We have characterized a tomato (*Lycopersicon esculentum*) gene encoding a typical HSP80 protein. This gene, called HSC80, is interrupted by two introns, 995 and 109 bp long. Northern blot analyses and in situ RNA hybridization show that HSC80 mRNA is abundant in shoot and root apices and in fertilized ovaries up to 6 d postanthesis but is rare in mature leaves. Heat shock increased mRNA levels in mature leaves but only 3-fold. Developmental regulation of the HSC80 gene was confirmed by fusing 2 kb of its 5' region to the β -glucuronidase reporter gene and introducing the chimeric gene into tomatoes. The roots of transformants showed high β -glucuronidase expression in the apex and in lateral root primordia but not in mature tissue. Expression in the shoot was up to 10-fold higher in the apex than in mature leaves. Thus, HSC80 is preferentially expressed in shoot and root apices during normal development.

HSPs⁴, which are conserved in all organisms, were originally thought to be protective factors induced specifically by heat stress. It was discovered subsequently that most HSPs are also developmentally regulated (18, 20) in the absence of stress. Isoforms of HSPs that are developmentally regulated are referred to as HSCs. HSP80 is a major HSP that is present in unstressed cells of yeast, *Drosophila*, and mammals. HSP80 has been called HSP82, 83, 84, and 90, depending on the electrophoretic estimate of the protein mass, but we shall use "HSP80" as a generic term (18).

HSP80 is indispensable. In yeast, it is encoded by two genes: one heat inducible and one expressed under normal conditions. Inactivation of either gene is aphenotypic at 25°C, but inactivation of both genes is lethal (3, 9). In *Drosophila*,

HSP80 expression is highest in the ovaries, and a single gene is responsible for both heat-shock and developmental regulation (30). In the absence of heat shock, HSP80s can constitute 2% of the soluble protein in the brain and gonads of mammals (18). HSP80s are found in both the nucleus and cytoplasm regardless of temperature and physiological condition (11, 18). It is interesting that a fraction of HSP80 is associated with other proteins. The most studied association of several identified is one with steroid receptors (5). Steroid receptors are transcriptional regulators activated by the binding of steroid hormones. In the absence of the specific ligand, the receptors are inactive and complexed with HSP80 and a 50-kD protein (18). Activation of the receptor by steroid hormones coincides with dissociation from HSP80. HSP80 is probably necessary for signal transduction in steroid hormone responses because depletion of HSP80 in yeast prevented steroid-mediated activation of a steroid-responsive gene (23). Other proteins associated with HSP80 are the Rous sarcoma virus pp60^{src} protein kinase (4), actin (17), and tubulin (26). These observations suggest that one role of HSP80 is to interact with other proteins, possibly stabilizing them and regulating their function.

There is limited information concerning developmental regulation of HSPs in plants. HSPs have been observed during embryogenesis (27). Developmentally regulated genes encoding HSP70 cognate proteins have been described in tomato (7) and in *Arabidopsis* (29). Is there a developmentally regulated HSP80 gene in plants? A cDNA encoding HSP80 has been cloned from *Arabidopsis* (6). This gene was expressed at a very low level in normal plants and was rapidly induced by heat shock. However, other HSP80 loci were detected by Southern blot analysis that might exhibit different regulation. In tomato cell culture, HSP80 was present in considerable amounts at normal temperature and increased 10-fold upon heat shock (21).

These observations and the evidence from other systems presented above suggest that plants may regulate HSP80 both developmentally and in response to heat shock. We report here the isolation and characterization of a tomato (*Lycopersicon esculentum*) gene encoding a developmentally regulated HSP80, which we call HSC80. The developmental regulation of this gene is demonstrated by northern blot hybridization, in situ RNA hybridization, and the expression pattern conferred by its promoter on a marker gene. We conclude that HSC80 is preferentially expressed in growing apices.

¹ Present address: Department of Zoology NJ-15, University of Washington, Seattle, WA 98195.

² Present address: Vollum Institute, L474, Oregon Health Sciences University, Portland, OR 97201-3098.

³ Present address: Department of Botany KB-15, University of Washington, Seattle, WA 98195.

⁴ Abbreviations: HSP, heat-shock protein; HSC, heat-shock cognate protein; SSC, standard sodium citrate; X-Gluc, 5-bromo-4-chloro-3-indolyl D-glucuronic acid; NPT, neomycin phosphotransferase; mas, mannopine synthase.

MATERIALS AND METHODS

Nucleic Acid Manipulations

Unless specified, we followed procedures described by Sambrook et al. (25). Construction and screening of the cDNA library has been described (16). Sequencing of DNA was by the use of the dideoxy chain termination reaction using a Sequenase sequencing kit (US Biochemical Corp., Cleveland, Ohio). All listed sequences were verified on the opposite strand. Primer extension analysis was carried out on mRNA isolated from tomato shoot apices (see below). The primer was complementary to the sequence CCAGGCTAGACAT-CAATCAGC, which initiates at nucleotide 168 of the sequence in Figure 2.

Southern and Northern Blot Analyses

Tomato plants (*Lycopersicon esculentum* var UC82B) were grown in growth chambers at 25°C, 8-h nights, 500 $\mu\text{E}/\text{m}^2$. Shoot tips (5-mm apical plumule), roots, and mature leaves were collected from plants 3 to 4 weeks old and immediately frozen in liquid N_2 . Tomato flowers and fruits were harvested from plants grown in a greenhouse, under 16-h light and 8-h dark periods, with temperatures fluctuating between 18 and 27°C. Flowers were marked at anthesis and harvested at the time specified. RNA was prepared from ovaries. Heat shock was induced in growth chambers with plants 3 to 4 weeks old by raising the temperature to 42°C and the RH to approximately 100% for 1 or 2 h. Despite the high humidity, transpiration cools plants in air, and plant tissue may not reach the air temperature. To ensure that heat-shock conditions had been achieved, a higher temperature, 55°C for 2 h, was also tested. Other studies (D. Alexander, unpublished observations) had revealed optimal induction of HSPs in tomato heat shocked at 55°C for 2 h. Leaves and shoot apices from heat-shocked plants were harvested immediately at the end of the 2-h treatment. Field plants were grown in a Yolo County (California) field under typical farming conditions and in temperatures from 15 (nighttime low) to 33°C (daytime high). Only leaves and apical shoots were collected from field plants.

For Southern analysis, tomato genomic DNA isolated as previously reported was used (16). The DNA was digested with various enzymes, and 5 μg of DNA per lane was electrophoresed on an agarose gel and blotted onto Zetaprobe nylon membranes according to procedures recommended by the manufacturer (Gibco-BRL, Grand Island, NY).

Northern blots were performed according to the protocol described previously (16). RNA was extracted using a modified guanidinium thiocyanate method (16). Polyadenylated RNA was purified using oligo(dT)-cellulose chromatography. Total RNA was used for the northern blots in Figures 4 and 5. RNA concentration was measured by spectrophotometric absorbance. Equal amounts were loaded in each lane. The loading was further verified by fluorescent staining of RNA in the gel with ethidium bromide. In these northern blots, concentration standards consisted of DNA segments obtained from digestion of HSC80 cDNA clones. Size standards were end-labeled λ -phage DNA *Hind*III fragments. The size estimate and approximate abundance of the HSC80 mRNAs

were confirmed by additional northern analyses performed on polyadenylated RNA and using RNA size standards. For RNA detection, two probes were used. The coding region probe spanned from amino acids 153 to 416 and was prepared by gel purification of an *Asp*718 fragment from the cDNA cloned in plasmid pCGN7100 (see "Results"). This fragment corresponded to the one from nucleotides 1758 to 2551 of the sequence in Figure 2. The 3' probe was generated by the polymerase chain reaction. The first primer spanned nucleotides 3332 to 3351, and the second spanned nucleotides 3524 to 3504. DNA probes were labeled with the Gibco-BRL nick translation kit. Hybridizations were in a solution containing 50% formamide, 1 M NaCl, 1% SDS, and 100 mg/mL of sheared salmon sperm DNA, at 22 to 37°C for low-stringency conditions or at 42°C for high-stringency conditions. Low-stringency washes were in 2 \times SSC, 0.1% SDS at 37°C, whereas high-stringency washes were at 68°C in 0.2 \times SSC, 0.1% SDS. Signals in northern blots were compared by image analysis of x-ray film negatives, using a Biological Vision (Berkeley, CA) image analyzer apparatus.

In Situ RNA Hybridization

The cDNA sequence in pCGN7100 was excised from the vector using *Xba*I and ligated into pBluescript II SK (Stratagene, San Diego, CA), also cut with *Xba*I, to create pCGN7104. In this vector, use of T7 or T3 RNA polymerases results, respectively, in transcription of sense or antisense HSC80 RNA. For in situ hybridizations to sections of tomato shoot tips and roots, ^{35}S -labeled RNA transcripts were used (16). Hybridizations were at 37°C. Hybridized sections were washed for 20 min each sequentially in the following buffers: at 37°C, 2 \times SSC, 50% formamide, then 37°C, 1 \times SSC, 50% formamide, and finally at 30°C, 1 \times SSC without formamide.

Construction of HSC80-*gusA* Chimeric Gene

The λ -Charon35 genomic DNA library of tomato was kindly provided by Dr. Robert Fischer (University of California, Berkeley). This library was screened with pCGN7100, and several clones of the A and B loci were isolated. Clone gHSP4 containing the HSC80 (A) locus was digested with *Eco*RI, and the resulting fragments were cloned in pUC18. A 5219-bp fragment contained the 5' end of the gene from an *Eco*RI site at -2070 from the start of transcription to an *Eco*RI site within the coding region. A unique *Bgl*III site was located at +58. The *Bgl*III to *Eco*RI fragment containing the region from +58 to -2070 was cloned into the *Bam*HI to *Eco*RI sites of pBluescript II SK. The *Bgl*III/*Bam*HI hybrid site is immediately flanked on the vector site by an *Xba*I and an *Spe*I site. An *Asp*718 site is located on the other side of the cloned fragment. The HSC80 5'-promoter region was excised as an *Asp*718 to *Spe*I fragment and cloned into a vector called pCGN7398, cut with *Asp*718 and *Xba*I. This vector contains the *gusA* locus (15) and the 3' region of the *mas* locus (nucleotides 19,239-18,474 [2]). A polylinker containing *Asp*718 and *Xba*I is located to the 5' of the *gusA*-*mas* 3' region. Cloning of the HSC80 5' region in that polylinker resulted in a chimeric gene in which transcription would initiate in the HSC80 5' region, continue through the *gusA*-

coding region, and terminate in the *mas* 3' region. This chimeric gene was called *HSC80-Gus*, and it is flanked by *XhoI* sites.

Plant Transformation

The *HSC80-Gus* chimeric gene was contained in an *XhoI* fragment. To generate a *PstI* fragment, we used a converter plasmid in which an *XhoI* site is flanked by *PstI* sites. After cloning the *HSC80-Gus* gene as an *XhoI* fragment in the converter plasmid, we excised with *PstI* and cloned it into the binary vector pCGN1547 (19). We chose the orientation in which the *HSC80-Gus* is transcribed in the same direction as the NPT gene, i.e. from the left T-DNA border toward the right T-DNA border, and called the construct pCGN7129. The binary vector pCGN7129 was introduced into *Agrobacterium tumefaciens* LBA4404 (13) by transformation. *L. esculentum* var UC82B was cocultivated as described by Fillatti et al. (10). To ensure independent transformation events, we propagated only individuals from different explants. Each transformant was given a number consisting of the binary plasmid used, followed by a hyphen and a progressive number (e.g. 7129-1, 7129-2).

Gus Assay and Histology

Transformed T₁ plants generated by *Agrobacterium* cocultivation were potted in soil mix and grown in a greenhouse to obtain seed. The analyses were confined to T₂ individuals, i.e. the progeny of T₁ plants. Seeds were surface sterilized in 70% ethanol for 1 min followed by 5 min in 50% commercial bleach, 0.1% Tween-20. They were rinsed five times in sterile distilled water and plated on half-strength Murashige-Skoog salts solidified with 0.8% Phytagar (Gibco) in 10- × 1.5-cm Petri dishes. The seeds were germinated at 23°C under fluorescent lights on a 16-h day period. The tap root apex (5–10 mm) was removed from the seedling with a scalpel about 1 week after germination. At that time, the cotyledons were fully enlarged, and the primordium of the first leaf was visible. The removed root apex was stained for Gus activity by the protocol of Jefferson et al. (15) using X-Gluc. Some of the positive seedlings were allowed to heal for 1 week, transplanted into potting mix, and grown in a greenhouse. These plants were tested for Gus activity by the fluorimetric assay based on 4-methylumbelliferyl D-glucuronide hydrolysis (15). Extracts were prepared from either the vegetative or the flowering shoot apex. The vegetative apex included a leaf primordium of 4 to 5 mm. The flowering apex also included the same size leaf primordium and consisted of a cluster of flower buds (a sympodium) smaller than 4 mm.

We also sampled leaves at different developmental stages. Leaf 1 was approximately 10 mm long, leaf 2 was about 25 mm long, and mature leaves were 10 to 30 cm long. Heat shock was induced by subjecting a Petri dish with several seedlings or whole potted plants to 42°C for 2 h. Histological assay of seedlings was initiated immediately after heat shock. Plant parts for enzyme extraction were harvested immediately after heat shock induction, flash frozen in liquid nitrogen, and stored at -85°C until assayed. *sd* values were calculated from the activities of three different samples of

comparable tissues harvested from the same individual. In each case, it was found to be less than 10%. Duplicate assays of the same enzyme preparation gave virtually identical readings.

RESULTS

Isolation and Characterization of a Tomato HSC80 Gene

We previously described construction and screening of a tomato apical shoot cDNA library (16). Several clones that hybridized strongly to root and shoot tip cDNA probes and little, or not at all, to a mature leaf probe were characterized. One of these, clone pCGN7100, was 1.1 kb long and encoded a protein with strong similarity to yeast and fruit fly HSP80. We subjected the tomato genome to Southern blot analysis to determine the number of loci coding for HSP80. Tomato DNA digested with *Asp718*, *BamHI*, *BglII*, and *HindIII* was blotted and probed with pCGN7100. Two bands in each digest hybridized with the intensity expected from a single-copy gene. Because one of the two bands appeared to hybridize less intensely to the probe, we hypothesized that there may be two loci, one of which corresponded to our cDNA.

Using the cDNA probe, we isolated several clones from a λ -Charon 35 genomic library of tomato. We characterized by restriction digestion and Southern blot analysis two types of clones corresponding to the two hybridization signals of the genomic Southern blot (Fig. 1). Sequencing of subcloned regions from both types of phages confirmed that two homologous loci exist. They were called, preliminarily, A and B. We concentrated on class A clones, which correspond to the original cDNA clone we had isolated.

Sequence analysis of clone 7100 revealed that it spanned a region from a site in the middle of the gene to a stretch of adenylate residues 188 nucleotides 3' of the translational stop codon. There was no consensus eukaryotic polyadenylation motif (AAUAAA) in the 3' region, but that is not unusual for a plant gene (14). To obtain a longer clone, we used the 5'-terminal portion to rescreen the cDNA library. The longest cDNA clone found was 7115, 1.9 kb long. Size comparison to the mRNA signal in northern blots (2.3 kb) and alignment with yeast and fruit fly HSP80 amino acid sequences showed that the clone was missing part of the 5' region. The sequencing was continued on a genomic clone of the A locus, called A4. We found that the coding region in the 5' region of the gene was interrupted by two introns, 996 and 110 bp long. The entire genomic region was sequenced in both directions and is listed in Figure 2.

The nucleotide sequence similarity between the tomato and the *Arabidopsis* genes is high: depending on the region, residue identity varies between 55 and 85% (not shown). The sequences diverge outside the coding region. We mapped the mRNA start site by primer extension and direct RNA sequencing to about 70 bp upstream of the start codon (Fig. 2). A putative TATA box region is located 25 bp upstream, and it is preceded, in the -40 to -80 region, by a fair match to the heat-shock element consensus (1, 22). Sequence analysis of an additional 2 kb of the 5'-promoter region failed to disclose another good match to the heat-shock element consensus (A.J. Koning, R. Rose, L. Comai, unpublished results).

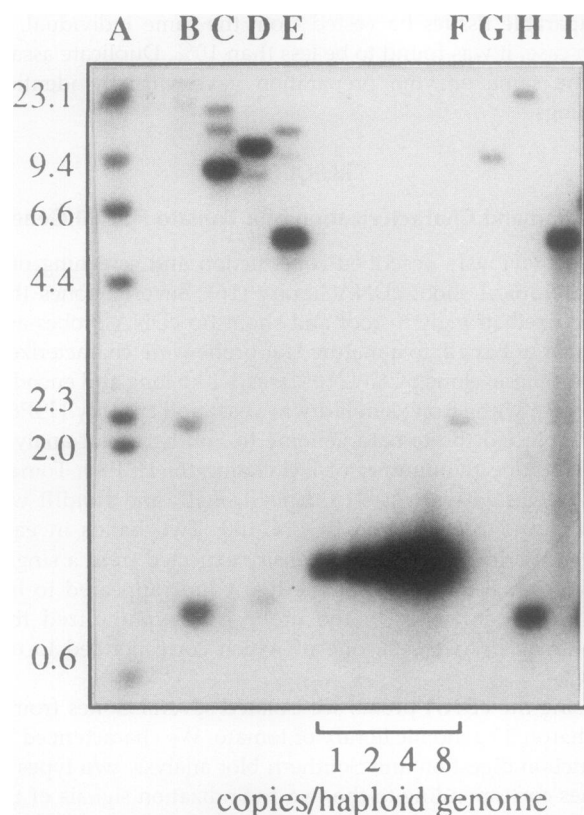


Figure 1. Analysis of HSC80 hybridization to the tomato genome. The Southern blot was probed with cDNA clone pCGN7100 representing the carboxy-terminal half of the HSC80 coding region. A, λ -Phage DNA *Hind*III molecular size markers in kb shown at left. B to E, Tomato DNA digested with *Asp*718 (B), *Bam*HI (C), *Bgl*II (D), *Hind*III (E); F, λ -phage clone B7 digested with *Asp*718; G, λ -phage clone B7 digested with *Bam*HI; I, clone A4 digested with *Asp*718; J, clone A4 digested with *Bam*HI. The four unlabeled lanes in the center are a copy number reconstruction using the cDNA in pCGN7100. The A4 clone carries the *Asp*718 0.8-kb fragment (lane H), and the B7 carries the *Asp*718 2.2-kb fragment (lane F), proving, together with the sequence data in Figure 2, that the two fragments (lane B) correspond to separate loci. The appearance of a third band in lane C is due to partial digestion by *Bam*HI (our unpublished results).

Only 120 bp of the B locus were sequenced. Its nucleotide sequence was 88% identical with the corresponding region of the A locus, and the encoded peptide, from position 619 to 659, differed only in a single residue (Glu⁶⁵⁰ to Asp) from the corresponding A-encoded peptide. Although the comparison is limited, it strongly suggests that the B locus encodes a second HSP80 protein. Because of the developmental expression pattern of the A locus (see below), we named it HSC80.

The sequence of the tomato protein is typical of all previously characterized eukaryotic HSP80s. It has a predicted mol wt of 80,500 with 65% amino acid residue identity to yeast HSP80, 71% to *Drosophila* HSP80, and 88% to *Arabidopsis* HSP80. Like other HSP80s, the tomato protein contains a highly charged region composed essentially of basic and acidic residues between amino acids 210 and 270. Figure

3 shows the amino acid sequence comparison to *Arabidopsis* HSP80.

Expression of the HSC80 Gene

We investigated the tissue distribution of HSC80 mRNA in tomato by northern blot analysis and found that this mRNA was very abundant in roots and in shoot apices (Fig. 4). By comparison to concentration standards (see "Materials and Methods"), it represented from 0.2 to 1% of the total mRNA in these organs. The abundance of HSC80 message in leaves was about 10-fold less than in apices. The higher levels in shoot apex than in mature leaf were found repeatedly using both growth chamber-grown and field-grown plants, indicating that this is a normal pattern rather than a stress-induced response. Heat shock had little effect on HSC80 expression in shoot apices because this treatment increased the level of mRNA by 50% at most. In mature leaves, however, heat shock did increase expression about 3-fold.

Because we detected two HSP80-type loci by Southern analysis and cloning, we wanted to determine the contribution of the A locus to overall gene expression. We used two different probes: a coding region probe that hybridized to both the A and the B locus and a 3' probe that contained the four carboxy-terminal codons plus the nontranslated, 3'-transcribed region. This A locus probe was specific, because it only hybridized to one band in genomic Southern blots (data not shown). Northern blot analysis indicated that the A locus is the major contributor to the observed expression of HSP80, because the A-specific probe and the coding region probe showed similar patterns of hybridization (data not shown). The B locus may or may not be expressed.

We next studied the expression of HSC80 in developing tomato fruits by northern blot analysis of RNA isolated from ovaries at different times after anthesis, which is also the time of fertilization. Equal amounts of total RNA from each developmental stage were subjected to electrophoresis, blotted, and probed with the same coding region probe used in Figure 4. As shown in Figure 5, the steady-state mRNA level is very high before and during anthesis and up to 6 d after anthesis. It decreases abruptly at the onset of ovary enlargement.

In situ hybridization analysis of HSC80 RNA provided information about the gene's histological expression pattern. Figure 6 shows the pattern of HSC80 mRNA distribution in root apices. High levels were detected in the apical 2-mm region representing the meristem. Lower levels were found in the root cap. The abundance of HSC80 mRNA decreased in the region 2 to 5 mm from the apex and was very low in the maturation zone of the root. Figure 7 shows the mRNA pattern in the floral apical shoot. Flower meristems exhibited high mRNA levels. The mRNA levels decreased basipetally along the young stem but remained high in the procambium and cambial region. In summary, expression of HSC80 can be observed in all young and dividing cells, whereas mature cells have a low expression.

*** **

```

CCCATAGAGAG GATCTAGAAA CCTAATAAG GCTAGTATAT AAGCTCGCTA AAAACTTCCC TTGATCCTCC
TCTTGCATTC GAGTCCCTTT GTGTTCATTT TCGCCTTACA GTTTTCTTCA GCTCTTTAGA TCTACAAAAA
A
142 ATG TCG GAC GTA GAG ACG TTT GCT TTC CAG GCT GAG ATC AAT CAG CTT CTC AGT
H S D V E T F A F Q A G A T I N Q L L S
196 CTT ATC ATC AAC ACT TTC TAC AGC AAC AAG GAG ATC TTT CTC CQT GAA CTC ATC
L I T L M T F Y S N M K E I F L R E L I
250 AGC AAT TCT TCC GAT
S N S S D
GTAAGTCTCC GAGCGTTTAG TTTTITTTAT CTCTAGGAT ACTGTGGTIT TACTGTCTIT TCATAGTTTA
AAATTTGATT ATTCAGAGC GATCTGAGCT CTTTITTTAT GCTTCGCGAG AAAGCTTAAC GTGATCATG
TGATTTTGA GTTTGATTAT TAGAGAGCAG CATCAATTTT TTTTGGTIT TTATAGTAT TTGTGGTGTG
TATAGCTTAA CGTGATCTTC TAGGATAGCTG TAGTGCTAT ATTTTGGAG CAATGATTA TTTAGATCAG
TTTATTTCT ATGTGGAGGT TATATGCTTA TCTGTATTGT TGTGTGGAT AGCTTAAAGT GATCTCCAG
CATACACTGT AGTGTATGCG CCTCTTTGA TTAAGAAGCTG TGATATTTT AGAGCAGCTG AGTATTTTG
TGAGTTTTT CATATGTAIT CTATATATGG ATAGCTTGTAT GTGATTGTCT GGGATAGTCC TGTCCACAG
TCTCTGTCTT GGATCGTTCA GAGCTCTGTT TTTGGCATCT TAATCTTAGT GTGATAGGAG TGAGTATTTA
GCCTGCCGCTG TATCTCCAAT GGTAAITAAA AATTGCGCT GTTGTGTAT CGTCTCTCT AGATACCITAG
TCTGAACATA TGTATCATCG TAGTGTAAAT TATATGATT TCACTATGAT ATACCATAGT GCCATCGACC
TCTGTGACTG CGCAAAATAA TTTGAGTAAT TTAGAGCACC TAATTCAGAT TTTGCTTTG TCGTCATAAT
AAGAGTTTTT CTAGTGTGCC TATCTCTGTT CAGACTAGGT AATCGAGATA CAGCTCTGAA TTTTGTATTG
CCGATCAGCT AATTAATCA ATATGTTTCT GGTGAGGGA TCGGTTCTG TTATCTGAT CTATATATC
ACAGGAATAG AATGTTTGT TCTTACTGCG TTGTAAATG TATGCGATAT TTGATATTT TTGTATTTT
GGATATATC TTTTATG
1261 GCT CTA GAC AAG ATC CGC TTT GAG AGT TTA ACC GAC AAG ACC AAG CTA GAT GGT
A L D K I R F E S L T D K S K L D G
1315 CAA CCA GAG CTC TTC ATC CAT ATT ATT CCA GAC AAG GCC AAT AAT ACT CTC ACT
Q P E L F I H I I F D K A N N T L T
1369 ATC ATT GAT AGT GGT ATT GGT ATG ACA AAG GCT G
I I D S G I G M T K A D
GTAAGTAGTA GGGACATATT GGAAGCTAGA AGCTCAACCA GAGTTTATC TATATATCT GTTCTTAATG
TTGCATTCT ATTGCTGAC CTTCCTTGT TTAITTCAG AT 1514
1515 CTG GTG AAT AAC CTG GGT ACA ATT GCA AAG TCA GGA ACC AAG GAG TTC ATG GAA
L V N N L G S G T I A R S S G T K E F M E
1569 GCT CTT GCA GCT GGT GCT GAT GTT AGC ATG ATT GGT CAA TTC GGT GTA GGT TTC
A L A A G A D V S M I G Q F V G F
1623 TAC TCT GCT TAC TTG GTA GCT GAG AAG GTT GTT GTG ACC ACA AAG CAC AAT GAT
Y S A Y L V A E K V K V T T K H N D
1771 GAT GAG CAA TAT GTC TGG GAG TCT CAA OCC GGT GGC TCT TTC ACT GTT ACC AGG
D E Q Y V N E S Q A G G S F T V T E
1637 GAT ACA TCT GGT GAG AAC CTT GGT AGG GGT ACC AAG ATG GTC CTT TAT CTC AAG
D T S G E N C L G R G T K M V L Y L K
1785 GAG GAT CAG CTT GAA TAC TTT GAA GAA CTT AGG CTC AAG GAC CTG ATT AAG AAG
E D Q L E Y L E E R R L K D L I K K
1839 CAC TCT GAG TTC ATT AGC TAT CCT ATT TCT CTG TGG GTT GAG AAG ACC ATA GAG
H S E F I S Y P I S L W V E K T I E
1893 AAG GAA ATT TCT GAT GAG GAG AAG GAA GAG AAG AAA GAT GAG GAG GAA AAG
K E I S D D E E E E K K D E E G K
1947 GTA GAG GAG GTC GAT GAG AAG AAG GAA GAG AAG AAA AAG AAG AAG GTC
V E E V D E E K E K E K K K K V
2001 AAA GAA GTT TCC AAT GAG TGG TCA CTG GTC AAC AAG CAG AAG CCT ATT TGG ATC
K E V S N E W S L V N K Q K P I W H
2055 AGA AAG CCA GAA GAG ATC ACA AAG GAA GAG TAT GCT GCT TTC TAC AAG AGC TTG
R K F E E I T K E Y A A F Y K S L
2109 ACT AAT GAT TGG GAA GAG CAT CTT GCT GTG AAG CAC TTC TCT GTT GAG GGT CAG
T N D H E H L A K H F S E G Q
2163 TTG GAG TTC AAG GCT GTC CTT TTT ATT CCA AAA AGG GCT CCT TTT GAC CTC TTT
L E F Y B V L F V P K R A P F D L E
2217 GAC ACA AAG AAG AAG CCC AAC AAC ATC AAG TTG TAT GTT CGC CGT GTC TTT ATC
D T K K K P N N I K L Y V R R V F I
2271 ATG GAT AAC TGT GAT GAG TTG ATT CPT GAA TAT TTG AGC TTT GTG AAG GGT ATT
M D N C D E L I P E Y L S F V K G I
2325 GTG GAT TCT GAG GAC CTT CCT CTC AAC ATC TCC AGA GAG ACA TTG CAG CAG AAC
V D S E D L N I S R E T L Q Q N
2379 AAG ATC CTA AAG GTT ATT CGC AAG AAT TTG GTG AAG AAG TGT GTT GAG CTT TFC
K I L K V I R K N L V K K C V E L F
2433 TTT GAA ATT GCT GAG AAC AAG GAG GAC TAC AAT AAG TTC TAT GAG CGC TCT TCT
F E I A E N K E D Y N K F Y E A F S
2487 AAA AAC CTC AAG CTT GGA ATC CAT GAG TAT TCT CAG AAC AAG GCA AAG TTT GCT
K N L K L G I H E D S Q N R A K F A
2541 GAA CTG CTG AGG TAC CAC TCC ACT AAG AGT GGT GAT GAG ATG ACC AAG TTG AAG
E L L R Y H D S T K S G D E M T S L K
2595 GAC TAT GTG ACC AGA ATG AAG GAG GGC CAG AAT GAT ATT TAC TAC ATT ACT GGT
D Y V T R H K E G Q N D I Y I T G
2649 GAG AGC AAG AAG GCT GTT GAG AAG TCT CCC TTC CTG GAG AAA CTG AAG AAG AAG
F S E Y R A L L F R L E K L F K N K
2703 GGA TAT GAG CTG CTT TAC ATG GTT GAT GCT ACC ATT GAT GAT TCA ATT GGT CAG
G Y E V L Y M V D A I D E Y S A I T G Q
2757 CTG AAG GAA TTT GAG GGC AAA AAG CTT GTT TCT GCT ACC AAG GAA GGC CTC AAG
L K E F E G K K L V S A T K E G L K
2811 CTT GAT GAG AGT GAA GAT GAG AAG AAA AAG CAG GAA GAA TTG AAG GAG AAG TTT
L D E S E D E K K K Q E E L K E K F
2865 GAG GGA CTG TGT AAG GTG ATG AAG GAT GTG CTA GGA GAC AAA GTT GAA AAG GTC
E G L C K V M K D V L G D K V E K V
2919 ATT GTT TCT GAC GCT GTT GTT GAC TCT CCC TGC TGT TTG GTC ACT GGT GAG TAT
I V S D R R V G D A S P C C L V T G E Y
2973 GGC TGG ACT GCT AAC ATG GAG AGA ATT ATG AAG GCA CAG GCA CTT AGG GAC TCC
G W T A N M E R I M K A Q A L R D S
3027 AAG ATG GCT GGA TAC ATG TCT AGC AAG AAG ACC ATG GAG ATC AAC CCA GAG AAC
S M A G Y N S S K K T M E I N P E N
3081 TCC ATC ATG GAT GAG CTA AAG AAG GGT GAT GAT GCA GAC AAG AAT GAG AAG TCT
S I M D E L R K R A D K N D K S
3135 GTG AAG GAC TTG GTT CTC TTG CTT TTT GAG ACT GCC CTT CTC ACC TCA GGT TTC
V K D L V L L L F R L E K L F S G F
3189 AGC CTC GAG GAG CCA AAC ACC TTT GGC AAC AGA ATT CAC AGG ATG TTG AAA CTC
S L E E P N T F G N R I H R N L K L
3243 GGT TTG AGC ATT GAT GAG GAA AGC GGA GAT GCT GAT GCT GAC ATT CCA GCA TTG
G L S I D E E S G D A D A D M P A
3297 GAG GAT CCT GAA GCT GAT GCT GAG GGC AGC AAG ATG GAG GAG GTT GAT TAA
E D P E A D A E G S K M G E V D
GTTCAATAA GTTTGATAG TTTTATGGGT TCTTTACTA CTACTTTAAT CCTAGTTTT TGCITTAATC
CATCAGAAC AATAGTGAGG GTTTTAATGC CGTTCITTTA GAATGGCAGT TCAATGTTAG GTTATAATC
TCTTTTGTG TTGACATTC GTGGTTGATA TAGTITTTT TCTGCC
3535

```

Figure 2. Nucleotide sequence of the HSC80 locus and amino acid sequence of the putative encoded protein shown in one-letter

Expression of Gus under the Control of the HSC80 5' Region

To assess the contribution of the 5' region of the HSC80 gene to expression and to confirm the above-reported expression pattern of this locus, we constructed a fusion of the HSC80 promoter region to the Gus reporter gene encoding β -glucuronidase (Gus, encoded by *gusA*, previously *uidA*; ref. 15) and studied its expression in plants. We subcloned a 2130-bp *EcoRI* to *BglIII* fragment encompassing the +60 to -2070 region from the start of transcription. This region was placed in front of the Gus reporter gene and the transcription termination region from the *mas* gene was placed 3' to the Gus gene. The chimeric gene, designated *HSC80-Gus*, was introduced into a binary vector, the vector was transformed into *A. tumefaciens*, and the resulting bacterial strain was used to generate transgenic tomato plants by cocultivation of cotyledonary explants. The structures of the HSC80 locus, the HSC80-Gus gene, and the binary vector T-DNA are shown in Figure 8.

Seed was collected from the primary transformants (T_1) and used in the following characterization. Seven independent transformant families were studied; these were the groups of siblings (T_2) produced by selfing each T_1 plant. We first characterized the expression of *HSC80-Gus* in the root. About fifteen seeds per family were surface sterilized and germinated on agar. Root tips were excised from the seedlings and stained with the indigogenic substrate X-Gluc. Figure 9 shows the root of a seedling from the 7129-10 family and provides an example of the observed expression pattern. All roots tested accumulated blue indigo dye in the apex. Expression was concentrated in the apical 1 mm corresponding to the root meristem and decreased rapidly to nondetectable levels in the root elongation and maturation zones. Some expression was evident in root cap cells but at lower levels than in the meristem. Mature roots did not stain. However, lateral root primordia stained as intensely as did the main root apex. Figure 9 shows two early primordia exhibiting strong *HSC80-Gus* expression. These primordia are at the very early stage of formation corresponding to proliferation of initials from the tap root pericycle. As primordia emerged from the primary root and developed into morphologically mature roots, their staining pattern was the same as that of the tap root apex.

Visual comparison of the different families allowed their classification as strong, medium, and low expressers, depending on the intensity of the stain. Variation among families was much greater than variation within each family, despite the expected segregation of each T-DNA in homozygotic and heterozygotic siblings. Therefore, interfamilial differences are

codes. The nucleotides where mRNA initiation was mapped are underlined (beginning of second line). We could not determine accurately which of the two nucleotides is the initiation site. The region with good homology to the TATA box motif has been marked by asterisks located above the nucleotide symbols. A region with good homology to the heat-shock regulatory element (1, 22) is also shown marked with lines above the nucleotide symbols. This entire sequence has been deposited in GenBank with the accession No. M96549.

seedlings of the 7129-4 family, in which the pattern of indigo accumulation was meristem specific but variegated. Variegation was manifested within each apex by the appearance of nonstaining longitudinal sectors.

Heat-shock treatment was administered to seedlings by exposure to 42°C for 2 h. This treatment resulted in decreased intensity of staining in the apices when compared with control seedlings kept at 25°C, but did not cause different or stronger staining of any other seedling part.

Some of the Gus-positive seedlings were transplanted and grown in a greenhouse. Different parts of these plants were then assayed for Gus expression by the fluorogenic assay using the substrate 4-methylumbelliferyl D-glucuronide. The highest specific activity was measured in shoot apices, but in some plants considerable activity was found in mature leaves as well. Remarkably, we found very large quantitative differences between different transformants. Figure 10 (A and B) shows Gus activities in three transformants: 7129-10 and 7129-8 are members of families exhibiting high-level expression of *HSC80-Gus* in roots, whereas in family 7129-14, medium-level expression was found. However, 7129-10 exhibited high-level expression in the shoot, whereas both 7129-14 and 7129-8 exhibited 1000-fold lower expression in this tissue. Heat shock was induced in these plants by subjecting them to 42°C for 2 h, but expression of *HSC80-Gus* in mature leaves did not increase (Fig. 10C).

DISCUSSION

We have isolated a tomato gene encoding a protein closely related to HSP80 of yeast, *Drosophila*, and *Arabidopsis*. The degree of amino acid sequence identity to these proteins is

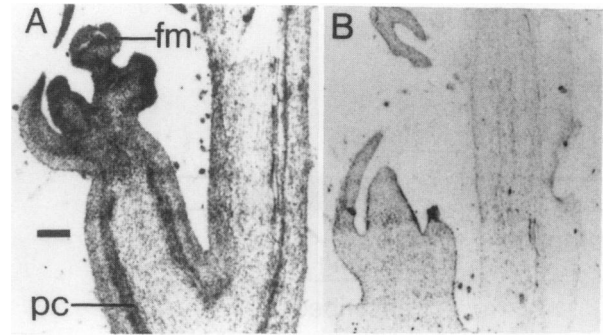


Figure 7. Localization of HSC80 mRNA in tomato floral apex. A, In situ mRNA hybridization of an HSP80 antisense probe to fixed sections of tomato shoot apex. The dark granular material is formed by silver grain, and its presence indicates hybridization and retention of the probe. The horizontal bar on the right corresponds to 100 μ m. B, Control hybridization with a sense HSC80 probe. The picture in B was overexposed to allow visualization of the plant section. fm, Floral meristem; pc, procambium.

very high: 65% to yeast HSP80, 71% to *Drosophila* HSP80, and 88% to *Arabidopsis* HSP80. This indicates that the protein encoded by the tomato gene belongs to the HSP80-HSP90 family. We designated this gene HSC80 to emphasize its developmental regulation rather than its heat-shock regulation. Southern analysis and genomic cloning also identified a second locus, the B locus, putatively encoding an HSP80.

Several observations indicate that the HSC80 gene, corresponding to the A locus, is expressed in a developmentally regulated fashion. First, we isolated tomato HSC80 by differ-

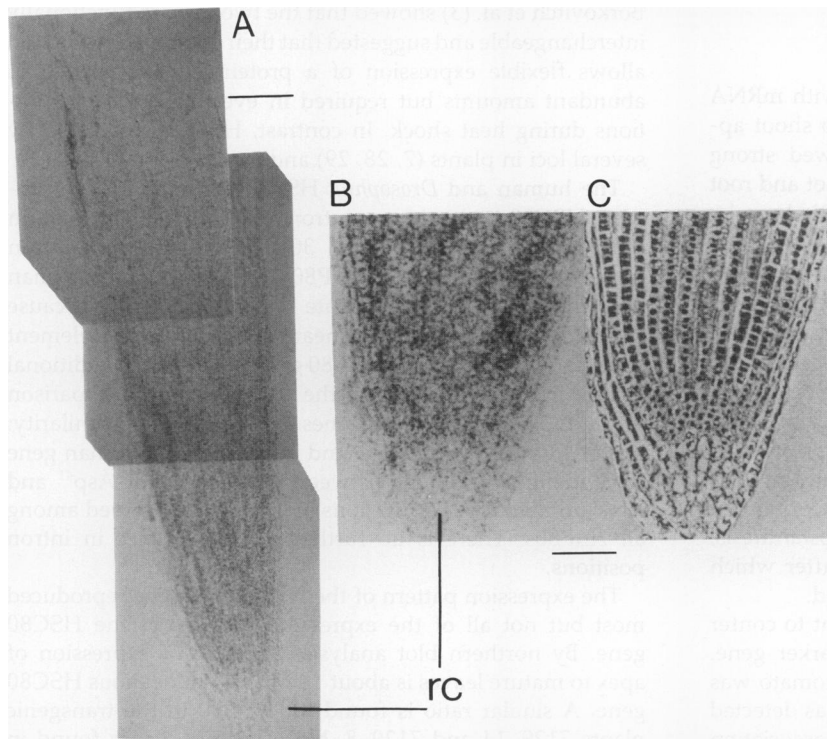


Figure 6. Cytological localization of HSC80 mRNA in a tomato root tip. Antisense RNA labeled with 35 S and complementary to HSC80 mRNA was hybridized to a median longitudinal section of a root apex from a seedling primary root. A, View from root cap (rc) to elongation zone by interference optics. The bar corresponds to 70 μ m. The distribution of black silver grains localizes HSC80 mRNA to the apical 1 mm. The intensity of the signal decreases to background above the shown region. Transverse sections 1 cm from the root apex show accumulation of silver grains in the pericycle (data not shown). Control hybridizations using a sense HSC80 probe showed negligible signal (see Fig. 7). B, Enlarged bright-field optics view of the root tip showing root cap and meristem. The bar corresponds to 35 μ m. C, Same as B but viewed by phase contrast optics.

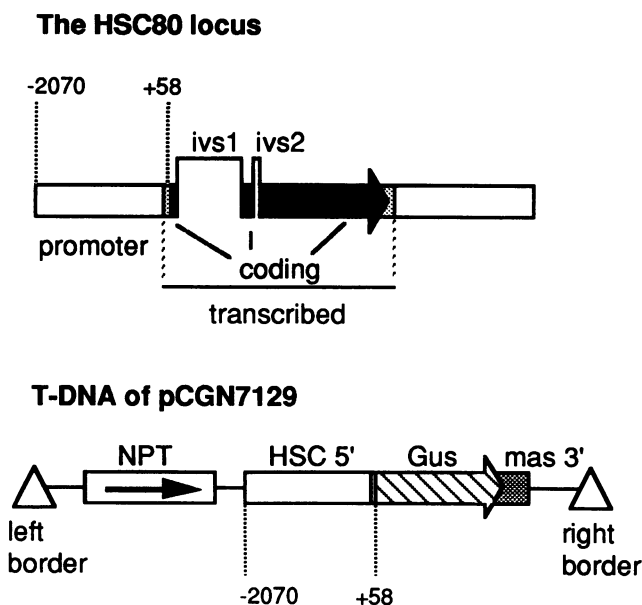


Figure 8. Structure of the HSC80 locus, of the *HSC80-Gus* chimeric gene, and of the T-DNA in the binary vector pCGN7129. In the HSC80 locus, the white boxed regions represent nontranscribed DNA, the stippled regions represent transcribed, noncoding DNA, the black regions represent coding DNA, and the raised lines represent two introns, labeled "ivs1" and "ivs2," respectively. The numbers in the 5'-promoter region define nucleotide positions relative to the point of transcription initiation. In the drawing of the T-DNA of pCGN7129, the only region derived from the HSC80 locus is the promoter, identified by the numbers -2070 to +58. The NPT gene consists of the mas promoter, the NPT gene, and the mas termination region. The arrow inside the NPT box shows direction of transcription. Details of the binary vector used (pCGN1547) can be found in the paper by McBride and Summerfelt (19).

ential screening of a cDNA library constructed with mRNA purified from normal (non-heat shocked) tomato shoot apices. Clone pCGN7100, encoding HSC80, showed strong hybridization to cDNA probes prepared from shoot and root apex mRNAs and weaker hybridization to a cDNA probe prepared from mature leaf mRNA.

Second, northern blot hybridization and in situ RNA hybridization analysis of HSC80 expression in several organs indicate that this gene is preferentially expressed in apices. In tomato roots, HSC80 mRNA is abundant in all cells of the apical first millimeter except those in root caps. In the mature root, hybridization is only found in the cambial region. Both vegetative and floral shoot apices show strong expression of this gene, whereas little expression is seen in maturing and mature cells. After fertilization and during fruit formation, we observed strong expression until day 6 postanthesis, coinciding with a phase of rapid cell division, after which time the steady-state level of the mRNA decreased.

Third, 2 kb of the HSC80 5' region is sufficient to confer developmental regulation on a heterologous marker gene. The *HSC80-Gus* chimeric gene transformed into tomato was expressed preferentially in shoot and root apices, as detected by histochemical and fluorogenic Gus assays. The association

with proliferating cells is exemplified by the strong histochemical reaction in developing root primordia, which turn dark blue, whereas the neighboring mature tissues show no histochemical reaction.

Fourth, two features of the HSC80 gene have been found to be usually associated with developmentally regulated heat-shock cognate genes rather than heat-shock-regulated genes: the presence of two introns in the transcribed region and the presence of a single consensus heat-shock element in the promoter region (12).

The presence of a single heat-shock element in the promoter may explain the increase in steady-state mRNA concentration after heat shock. Whereas this increase is detectable, it is small in comparison to the large induction observed for HSP80 in *Arabidopsis* (6). A second gene (the B locus) encoding a putative protein of the HSP80 type was also cloned from tomato. It is possible that this locus may be predominantly heat shock regulated. Our data provide no information about its expression because of the stringent hybridization conditions used in our northern analyses. Figure 1 shows that under stringent hybridization conditions the B locus hybridizes weakly to the HSC80-coding region probe and, therefore, would contribute proportionately less than HSC80 to a northern hybridization signal. Because cultivated tomato is inbred, it is unlikely that the A and B clones represent alleles of the same gene. Low-stringency hybridization of the HSC80 probe to tomato DNA failed to detect additional loci.

Two HSP80 genes are present in yeast, a single HSP80 is present in *Drosophila*, and two HSP80 genes and several HSP80-related proteins have been found in mammals (18). In yeast, one HSP80 locus is expressed during development and is referred to as HSC82, and the second, called HSP82, is induced to the level of the first only during heat shock. Borkovitch et al. (3) showed that the two loci are functionally interchangeable and suggested that their combined regulation allows flexible expression of a protein always needed in abundant amounts but required in even higher concentrations during heat shock. In contrast, HSP70 is encoded by several loci in plants (7, 28, 29) and in other organisms (18).

The human and *Drosophila* HSP80 genes differ from tomato HSC80 by having an intron, 1000 to 1500 bp long, in the 5'-noncoding region (24, 30). This is the only intron present in the *Drosophila* HSP80 gene. Intron 1 of human and *Drosophila* could participate in gene regulation, because it contains matches to the heat-shock regulatory element consensus. In the human HSP80 gene, there are 10 additional introns interspersed through the coding region. Comparison of the tomato and human genes shows a striking similarity: intron 1 of the tomato gene and intron 2 of the human gene have identical positions between the codons for Asp⁴¹ and Ala⁴² (tomato HSC80 positions) in a region conserved among all HSP80s. There is no further correspondence in intron positions.

The expression pattern of the *HSC80-Gus* gene reproduced most but not all of the expression features of the HSC80 gene. By northern blot analysis, the ratio of expression of apex to mature leaves is about 10 for the endogenous HSC80 gene. A similar ratio is found for HSC80 in the transgenic plants 7129-14 and 7129-8, but a ratio of 2 was found in

transgenic plant 7129-10. *HSC80-Gus* was not induced 2- to 3-fold by heat shock as was observed for HSC80 mRNA. These discrepancies could be due to the differences in the parameters measured, mRNA levels versus Gus enzyme activity, or to the absence of additional HSC80 regulatory elements in the *HSC80-Gus* construct. They do not alter the basic conclusion that the HSC80 gene is developmentally regulated. In future studies, we will test whether the intron-containing region or the 3' region of HSC80 have regulatory properties when incorporated in chimeric genes.

Why is HSC80 preferentially expressed in plant apices? HSP80 interacts with several proteins. The animal and the *Achlya bisexualis* homologs bind to steroid receptors. Steroids release the receptor from the complex with HSP80, allowing the receptor to function as a transcription factor (18). The interaction between steroid receptors and HSP80 has a regulatory effect, because depletion of HSP80 resulted in failure to activate steroid-regulated genes (23). Binding of specific factors by HSP80 could also occur in plants. Cells in shoot and root apices are poised to differentiate into mature tissues and organs. It is conceivable that HSP80 may form complexes with certain regulatory proteins designed to sense chemical or physical factors and activate a response or developmental path. The association of HSP80 with actin (17) and tubulin (26) may play a role in the assembly and disassembly (8) of

Table I. *HSC80-Gus* Expression Pattern in the Tap Root of Transgenic Tomato Seedlings

The analysis of each transformation event was carried out using the progeny of the original transformant. The 0- to 10-min apical section of the tap roots was excised from 2-week-old seedlings and incubated with the indigogenic Gus substrate X-Gluc. About 15 siblings were analyzed per transformation family. Regions of high Gus expression accumulated a blue indigo precipitate (see Fig. 9). The apical pattern corresponds to a positive response in the 1.5 mm of the apex and in lateral root initials. The variegated pattern corresponds to alternating positive and negative longitudinal sectors in the apex. All families exhibited light staining in root cap cells, with the exception of 7129-8 seedlings, which showed intense *HSC80-Gus* expression in the root cap. The intensity estimate was based on visual evaluation of several individuals per family.

Transformant No.	Root Staining with X-Gluc	
	Pattern	Intensity
7129-2	Apical	Medium
7129-4	Apical/variegated	Low
7129-5	Apical	Low
7129-7	Apical	Medium
7129-8	Apical + root cap	High
7129-9	Apical	High
7129-10	Apical	High
7129-14	Apical	Medium

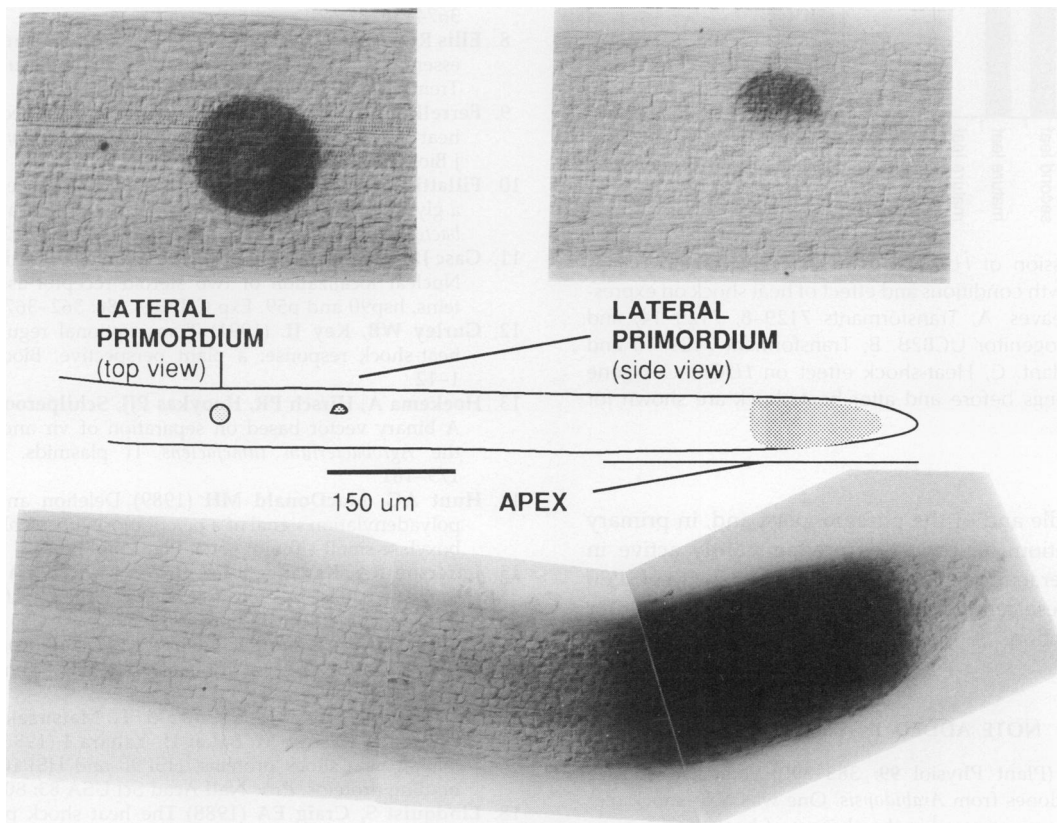


Figure 9. Expression of *HSC80-Gus* in the tap root of a transgenic tomato seedling. The T₂ seedling, the progeny of an original transformant, is one of the 7129-10 family (see "Results" and Table I). The photomicrograph was taken with Nomarski interference optics. Accumulation of dark indigo dye is visible in the root tip and in secondary root primordia, marking cells with high *HSC80-Gus* expression. Control plants showed no staining.

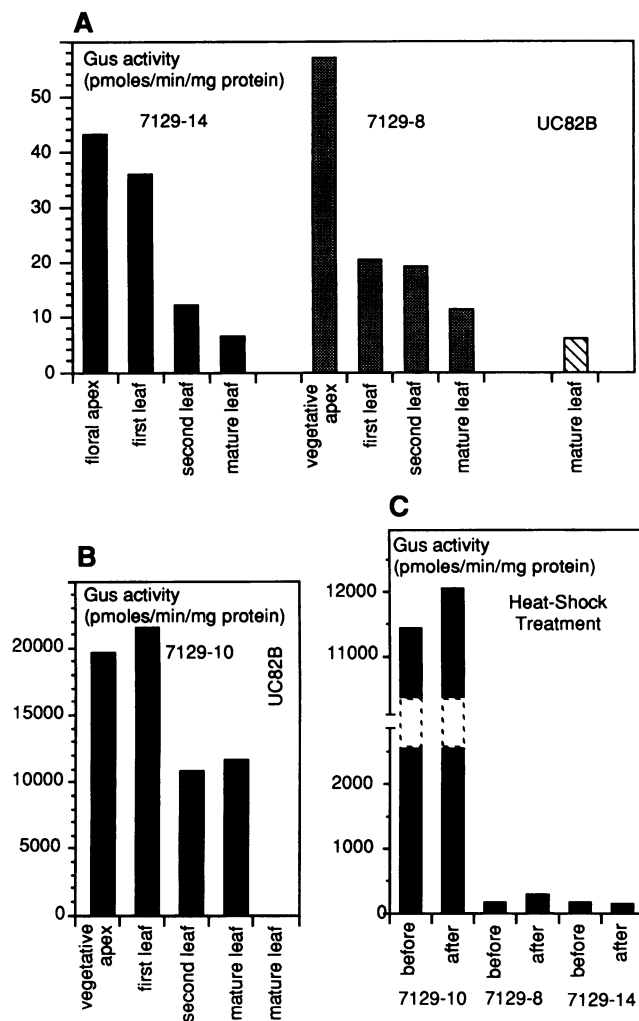


Figure 10. Expression of *HSC80-Gus* in different tomato organs under normal growth conditions and effect of heat shock on expression in mature leaves. A, Transformants 7129-8, 7129-14, and untransformed progenitor UC82B. B, Transformant 7129-10 and control UC82B plant. C, Heat-shock effect on *HSC80-Gus* gene expression. Readings before and after heat shock are shown for each transformant.

the mitotic spindle and of the phragmoplast and, in primary cell wall deposition, all processes predominantly active in plant apices. Interaction of cytoskeleton proteins with HSP80 may facilitate these cellular processes or be a requirement for cytoskeletal function.

NOTE ADDED IN PROOF

Takahashi et al. (Plant Physiol 99: 383-390) characterized two HSO80 genomic clones from *Arabidopsis*. One was heat-shock regulated, the other was expressed in the absence of heat-shock.

ACKNOWLEDGMENTS

We would like to thank Kristine Kiehne for help with the in situ histological analysis, Danny Alexander for help with the cDNA

library construction, Belinda Martineau for the blotted tomato fruit RNA, Kristin Summerfelt for tomato transformation, and Elizabeth Haswell for help with the analysis of transgenic plants. Finally, we are thankful for suggestions by Arnold Bendich and Greg Beaulieu concerning manuscript style.

LITERATURE CITED

- Amin J, Ananthan J, Voellmy R (1988) Key features of heat shock regulatory elements. *Mol Cell Biol* 8: 3761-3769
- Barker RF, Idler KB, Thompson DV, Kemp JD (1983) Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. *Plant Mol Biol* 2: 335-350
- Borkovich KA, Farrelly FW, Finkelstein DB, Taulien J, Lindquist S (1989) hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. *Mol Cell Biol* 9: 3919-3930
- Brugge JS, Erikson E, Erikson RL (1986) The specific interaction of the Rous Sarcoma virus transforming protein, pp60^{src} with two cellular proteins. *Cell* 25: 363-372
- Catelli MG, Binart N, Jung-Testas I, Renoir JM, Baulieu EE, Feramisco JR, Welch WJ (1985) The common 90-kd protein component of the non-transformed '85' steroid receptors is a heat-shock protein. *EMBO J* 4: 3131-3135
- Conner TW, LaFayette PR, Nagao RT, Key JL (1990) Sequence and expression of a HSP83 from *Arabidopsis thaliana*. *Plant Physiol* 94: 1689-1695
- Duck N, McCormick S, Winter J (1989) Heat shock protein HSP70 cognate gene expression in vegetative and reproductive organ of *Lycopersicon esculentum*. *Proc Natl Acad Sci USA* 86: 3674-3678
- Ellis RJ, Hemmingsen SM (1989) Molecular chaperones: proteins essential for the biogenesis of some macromolecular structures. *Trends Biochem Sci* 14: 339-342
- Ferrelly FW, Finkelstein DB (1984) Complete sequence of the heat shock-inducible HSP90 gene of *Saccharomyces cerevisiae*. *J Biol Chem* 259: 5745-5751
- Fillatti JJ, Kiser J, Rose R, Comai L (1987) Efficient transfer of a glyphosate tolerance gene into tomato using a binary *Agrobacterium tumefaciens* vector. *Biotechnology* 5: 725-730
- Gasc JM, Renoir JM, Faber LE, Delahaye F, Baulieu EE (1990) Nuclear localization of two steroid receptor-associated proteins, hsp90 and p59. *Exp Cell Res* 186: 362-367
- Gurley WB, Key JL (1991) Transcriptional regulation of the heat-shock response: a plant perspective. *Biochemistry* 30: 1-12
- Hoekema A, Hirsch PR, Hooykas PJJ, Schilperoort RA (1983) A binary vector based on separation of vir and T-region of the *Agrobacterium tumefaciens* Ti plasmids. *Nature* 303: 179-181
- Hunt AG, MacDonald MH (1989) Deletion analysis of the polyadenylation signal of a pea ribulose-1,5-bisphosphate carboxylase small-subunit gene. *Plant Mol Biol* 13: 125-138
- Jefferson RA, Kavanagh TA, Bevan MW (1987) Gus fusions: β -glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* 6: 3901-3907
- Koning AJ, Tanimoto EY, Kiehne K, Rost T, Comai L (1991) Cell-specific expression of plant histone H2A genes. *Plant Cell* 3: 657-665
- Koyasu S, Nishida E, Kadowaki T, Matsuzaki F, Iida K, Harada F, Kasuga M, Sakai H, Yahara I (1986) Two mammalian heat shock proteins, HSP90 and HSP100, are actin-binding proteins. *Proc Natl Acad Sci USA* 83: 8054-8058
- Lindquist S, Craig EA (1988) The heat shock proteins. In A Campbell, ed, Annual Review of Genetics, Vol 22. Annual Review Inc., Palo Alto, CA, pp 631-677
- McBride KE, Summerfelt KR (1990) Improved binary vectors for *Agrobacterium* mediated plant transformation. *Plant Mol Biol* 14: 269-276
- Nagao RT, Key JL (1989) Heat shock protein genes of plants. In

- J Schell, I Vasil, ed, Culture and Somatic Cell Genetics of Plants, Vol 6. Academic Press, New York, pp 297-328
21. **Nover L, Scharf K-D** (1984) Synthesis, modification, and structural binding of heat-shock proteins in tomato cell culture. *Eur J Biochem* **139**: 303-313
 22. **Pelham HRB** (1982) A regulatory upstream promoter element in the *Drosophila* HSP70 heat-shock gene. *Cell* **30**: 517-528
 23. **Picard D, Khursheed B, Garabedian MJ, Fortin MG, Lindquist S, Yamamoto KR** (1990) Reduced levels of hsp90 compromise steroid receptor action in vivo. *Nature* **348**: 166-168
 24. **Rebbe NF, Hickman WS, Ley TJ, Stafford DW, Hickman S** (1989) Nucleotide sequence and regulation of a human 90-kDa heat shock protein gene. *J Biol Chem* **264**: 15006-15011
 25. **Sambrook J, Fritsch EF, Maniatis T** (1987) Molecular cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
 26. **Sanchez ER, Redmond T, Scherrer LC, Bresnick EH, Welsh MJ, Pratt WB** (1988) Evidence that the 90-kilodalton heat shock protein is associated with tubulin-containing complexes in L cell cytosol and in intact PtK cells. *Mol Endocrinol* **2**: 756-760
 27. **Vierling E** (1991) The role of heat shock proteins in plants. *Annu Rev Plant Physiol Plant Mol Biol* **42**: 579-620
 28. **Winter J, Wright R, Duck N, Gasser C, Fraley R, Shah D** (1988) The inhibition of petunia HSP70 mRNA processing during CdCl₂ stress. *Mol Gen Genet* **211**: 315-319
 29. **Wu CH, Caspar T, Browse J, Lindquist S, Somerville C** (1988) Characterization of an HSP70 cognate gene family in *Arabidopsis*. *Plant Physiol* **88**: 731-740
 30. **Xiao H, Lis JT** (1989) Heat shock and developmental regulation of the *Drosophila melanogaster* hsp83 gene. *Mol Cell Biol* **9**: 1746-1753