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Three tomato genes code for heat stress transcription factors with a region of remarkable homology to the DNA-binding domain of the yeast HSF

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Heat stress (hs) treatment of cell cultures of *Lycopersicon peruvianum* (Lp, tomato) results in activation of preformed transcription factor(s) (HSF) binding to the heat stress consensus element (HSE). Using appropriate synthetic HSE oligonucleotides, three types of clones with potential HSE binding domains were isolated from a tomato λgt11 expression library by DNA-ligand screening. One of the potential HSF genes is constitutively expressed, the other two are hs-induced. Sequence comparison defines a single domain of ~90 amino acid residues common to all three genes and to the HSE—binding domain of the yeast HSF. The domain is flanked by proline residues and characterized by two long overlapping repeats. We speculate that the derived consensus sequence is also representative for other eukaryotic HSF and that the existence of several different HSF is not unique to plants.

Key words: heat stress/HSF gene/tomato transcription factor/yeast

Introduction

The heat stress (hs) response is observed in all types of organisms (Georgopoulos *et al.*, 1990; Lindquist and Craig, 1988; Nover, 1990). It involves a transient, complex reprogramming of cellular activities evidently needed to protect essential structures and functions against damage during the stress period and to provide optimum conditions for the recovery (Nover, 1990; Tomasovic, 1989). Central to the changing gene expression pattern is the massive synthesis of heat stress proteins (HSP) which belong to five families of conserved proteins (Lindquist and Craig, 1988; Nover, 1990; Nover *et al.*, 1990).

The basic structure of the heat stress control element (HSE) for transcriptional regulation of eukaryotic hs genes was originally described by Pelham (1982) and Pelham and Bienz (1982). It represents a palindromic element with repetitive purine and pyrimidine motifs: 5′-nGAAnnTTCnnGAAnnTTCn-3′ (Amin *et al.*, 1988; Nover, 1987; Schöffl *et al.*, 1988; Xiao and Lis, 1988). Different methods have been used for the characterization and purification of the postulated heat stress transcription factor (HSF) from *Drosophila* (Topol *et al.*, 1985; Wiederrecht *et al.*, 1988; Wu *et al.*, 1987), yeast (Sorger and Pelham, 1987, 1988; Wiederrecht *et al.*, 1988) and mammals (Goldenberg *et al.*, 1988). In all cases so far investigated, the HSF preexists under non-stress conditions but is activated by hs or chemical stressors. To date yeast is the only organism from which a HSF gene has been isolated and characterized (Sorger and Pelham, 1988; Wiederrecht *et al.*, 1988). The gene is essential for yeast and codes for a 93 218 dalton protein with a putative HSE binding and an oligomerization domain (Leu-zipper, for details see Figure 6). The native HSF of *Drosophila* and yeast are homotrimers (Perisic *et al.*, 1989; Sorger and Nelson, 1989). The activation by hs is associated with phosphorylation as was shown in *Drosophila*, yeast and mammals (Larson *et al.*, 1988; Sorger and Pelham, 1988; Sorger *et al.*, 1987; Wiederrecht *et al.*, 1988). Interestingly, the *in vitro* activation of the HSF from HeLa cells can be brought about not only by hs but also by low pH, Nonidet or urea, and this transformation of the HSF is inhibited by glycerol (Mosser *et al.*, 1990). These results suggest that changes of protein conformation are involved.

The results presented in this paper confirm the hs activation of DNA-binding of preformed HSF also in plants. South-Western screening of a tomato cDNA library in λgt11 led to the identification of three potential HSF genes. They all share a putative DNA binding domain of ~90 amino acid residues which is very similar to the corresponding region of the yeast HSF.

Results

Heat shock activation of tomato HSF

Similar to the results obtained with other eukaryotic organisms (Kingston *et al.*, 1987; Zimarino and Wu, 1987; Zimarino *et al.*, 1990), induction of hs genes in tomato cells is associated with the activation of a preformed HSE binding protein (Figure 1). The HSE-specific DNP complex is only formed when nuclear extracts from heat-shocked cells are used (Figure 1, part B lane 1 versus lane 2). Complex formation with the labeled HSB oligonucleotide is efficiently competed by a 32-fold excess of the unlabeled HSE oligonucleotide (lane 5 of Figure 1A) but neither by a mutant oligonucleotide (lanes 6–8) nor by an 800-fold excess of fish sperm DNA (lanes 3 and 6–8). Activation of tomato HSF binding is reversible and proceeds equally well in the presence of cycloheximide (Figure 1B). Induced HSE binding activity is observed after < 1.5 min hs at 39°C and is maximum after about 15 min (Figure 1C). Similar results were obtained using nuclear extracts from cell cultures of other plants, e.g. of *Digitalis lanata* (foxglove) and *Petroselinum hortense* (parsley).

Cloning of tomato HSF genes

One possible means for isolation of genes encoding DNA binding proteins is the screening of λgt11 cDNA expression libraries with a DNA ligand binding assay using specific oligonucleotides (Singh *et al.*, 1988, 1989). The success of this method depends on the maintenance of specificity and

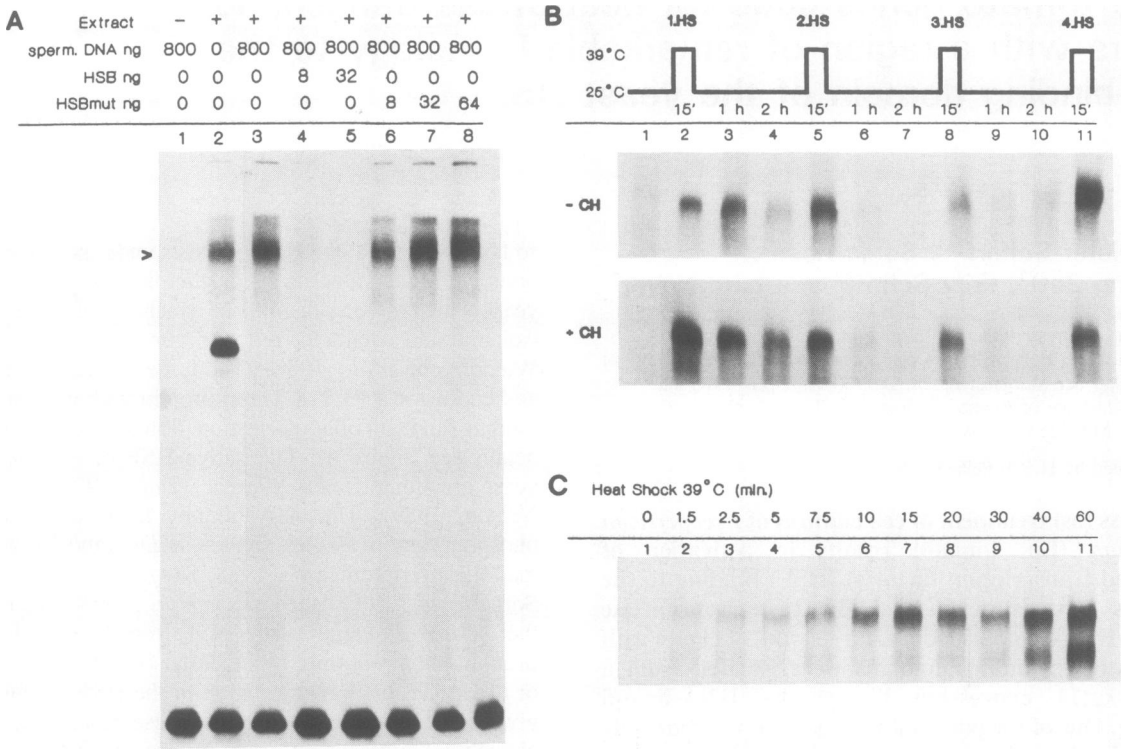

Fig. 1. Heat shock induced binding of tomato nuclear proteins to synthetic HSE oligonucleotides *in vitro*. Nuclear proteins were extracted from tomato cells and incubated with 1 ng of ^{32}P-labelled HSB oligonucleotide followed by gel retardation analyses (see Materials and methods). **A**. Specificity: The HSE-specific DNP complex (open arrowhead) disappears in the presence of excess unlabelled HSB (lanes 4,5) but not of mutant HSB (lanes 6–8) or 800 ng of salmon sperm DNA (lanes 3, 6–8). **B**. Reversibility: Repeated cycles of pulse activation (15 min, 39°C) and deactivation (2 h, 25°C) can be performed even in the presence of cycloheximide (CH). **C**. Time kinetics: Specific HSE-binding activity is observed after 1.5 min. The composition of the incubation mixture in B and C corresponds to lane 5 in part A. Both parts show only that region of the electropherogram with the specific DNP complexes, which sometimes form two bands (lanes 10, 11 of part C).

affinity of the corresponding DNA-binding domains as part of lacZ fusion proteins in *Escherichia coli*. In the primary screening of the cDNA library from tomato we used an oligomerized probe (HSB_n, see Materials and methods). cDNA libraries were prepared by random-priming starting with poly(A)$^+$RNA of control and heat-stressed tomato cells respectively. Out of a total of 2×10^6 plaques from both libraries, 64 positive clones were identified. After four rounds of purification, 23 clones remained positive, but only two of them derived from the control cDNA library. A characteristic of all of them is the capability of binding the HSB oligomer but not the mutant form. Eleven of these clones also reacted with the HSB monomer, and these were selected for further characterization (see example given in Figure 2).

Characterization of tomato HSF clones

Mutual cross hybridization of the 11 clones revealed three different genes. Two genes are represented by single clones, Lp-HSF8 from the control and Lp-HSF30 from the hs library, whereas the remaining 9 clones derived exclusively from the hs library and represent a third gene (Lp-HSF24). Their insert sizes range from 380 bp (subclone T37) to 850 bp (subclone T26). Due to the screening strategy, all inserts should contain the HSE-binding domain of the respective HSF. The binding capability of the lacZ fusion proteins for the three representative cDNA clones was verified in a gel retardation assay (Figure 3). Specific DNP complexes were detected with protein extracts from ITPG-induced lysogenic

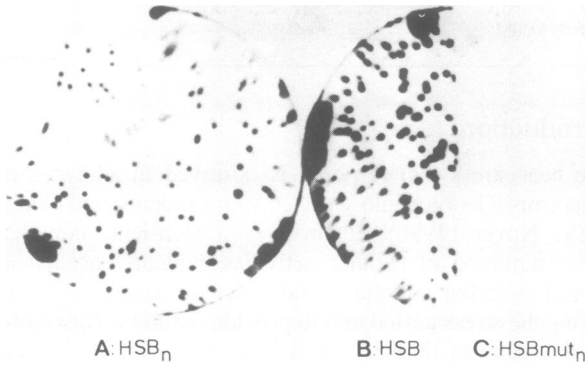

Fig. 2. DNA–ligand binding assay for the characterization of the purified HSF24-specific cDNA-clone using different hs oligonucleotide probes. A purified λ clone with insert T26 was amplified on a lawn of *E.coli* strain Y1090. Plaques from duplicate plates with identical inocula of bacterial cells were transferred to nitrocellulose, and the filters were processed as described for South-Western screening. For detection of binding activity, labelled probes of the oligomerized HSB_n (**A**), the HSB monomer (**B**) and the mutant HSB_n (**C**) were used.

E.coli cells containing the respective clones. Without induction (lanes 4–6) or with extracts containing only the native *lacZ* gene product (data not shown) radioactivity detected in the DNP region of the gel was negligible.

The individual character of the three HSF genes was further documented by the results of Southern and Northern hybridizations. For each gene a unique pattern of hybridizing

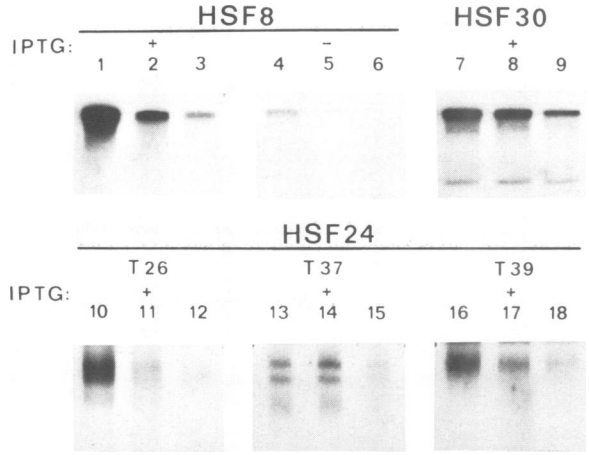

Fig. 3. Analysis of lacZ−HSF fusion proteins by gel retardation assays. Extracts of IPTG-induced, lysogenic *E.coli* cells (strain Y1089) were incubated with 1 ng of ^{32}P-labelled HSB oligonucleotide in the absence (lanes 1,4,7,10,13 and 16) or in the presence of 16 ng (lanes 2,5,8,11,14 and 17) and 32 ng (lanes 3,6,9,12,15 and 18) of unlabelled HSB oligonucleotide. Lanes 4−6 show an example of a lysogenic *E.coli* strain without IPTG induction. The particular type of HSF fragment is indicated on top. The lower part (lanes 10−18) shows the results for three different partial clones representing HSF24 (for details see Figure 6A).

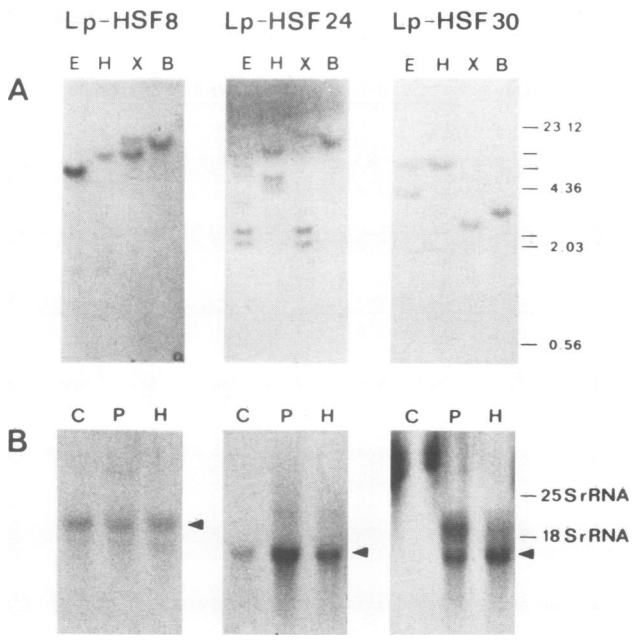

Fig. 4. Characterization of three types of HSF clones by Southern and Northern hybridization. **A**. Southern hybridization with genomic DNA of tomato digested with *Eco*RI, *Hin*dIII, *Xba*I and *Bgl*II respectively. Size markers derived from a λ DNA *Hin*dIII digest are indicated on the margin. **B**. For Northern hybridization total RNA was prepared from tomato cell cultures; control (C); preinduced for 15 min at 40°C plus 3 h at 25°C (P), and heat-shocked for 1 hr at 39°C (H). Each lane was loaded with 20 μg of total RNA. The positions of the two large species of the rRNA are marked.

restriction fragments of genomic DNA of tomato (Figure 4A) and a corresponding mRNA were identified (Figure 4B). Surprisingly, the Lp-HSF24 and Lp-HSF30 mRNAs are hs-inducible. In control cells, HSF30 mRNA is barely detectable at all. In contrast, Lp-HSF8 mRNA is constitutively expressed. It is essential to notice that each lane was loaded with the same amount of RNA. This was verified by ethidium bromide staining of the rRNA bands and by probing the same RNA samples with a constitutively expressed tomato clone (data not shown).

Based on the estimated size of their mRNAs (1.5 and 1.9 kb respectively) the tomato HSF are much smaller than their counterparts in yeast, *Drosophila* and mammals (Sorger and Pelham, 1988; Wiederrecht *et al.*, 1988; Zimarino *et al.*, 1990). Molecular weights of ~35 kd for HSF24 and HSF30 and of ~50 kd, for HSF8 were estimated (see results in Figure 5 for HSF24).

The HSE-binding domain

In agreement with the estimated size of the mRNA of 1.5 kb, a 1530 nucleotide clone of Lp-HSF24 was isolated from an oligo(dT)-primed cDNA library and sequenced (Figure 5). It contains a single open reading frame of 301 codons preceded by a long 5′-leader sequence of 327 nucleotides and followed by a 3′ untranslated trailer of 284 nucleotides. The putative HSF has a molecular weight of 33 300 daltons. Comparison of sequences derived from partial clones of the two other tomato HSF and the yeast HSF1 showed a striking homology in a region of 90−100 amino acid residues (Figure 6), which coincides almost precisely with the minimum clone T37 of the Lp-HSF24 (380 bp) and the DNA-binding domain identified for the yeast HSF1 (Sorger and Pelham, 1988; Wiederrecht *et al.*, 1988). A consensus sequence derived from the five clones (Figure 6B) very likely contains the basic elements of the eukaryotic HSE-binding domain.

Another interesting feature of HSF24 is a Leu-zipper type of regularly spaced hydrophobic amino acid residues (Figure 5, circles), which may be part of a putative oligomerization domain. The two parts of the protein are separated by a hydrophilic region of 58 amino acid residues with 18 serine or threonine and 5 proline residues.

Discussion

An important outcome of the South-Western screening of tomato cDNA libraries was the identification of three independent genes coding for putative heat stress transcription factors (see summary in Table). Although direct activity tests with the intact proteins are lacking, there is good evidence that these clones represent functional tomato HSF genes. First, their isolation from cDNA libraries and the Northern hybridizations (Figure 4B) document the expression of these genes. Moreover, the hs inducibility of clones HSF24 and HSF30 can explain why 10 out of 11 clones were isolated from the hs cDNA library. Second, the specificity and high affinity of interaction with the intact but not with the mutant HSB is in good agreement with the results of the gel retardation assays (Figure 1) obtained with native HSF from nuclear extracts. Third, the activity of the smallest partial clone T37 of 380 bp defines the minimum length of the HSE-binding domain. It is largely formed of the DNA-binding domain also found in the other two genes and in the yeast HSF gene (Figure 6, Sorger and Pelham, 1988; Wiederrecht *et al.*, 1988). Interestingly, Jakobsen and Pelham sequenced the HSF gene of Kluyveromyces lactis, which is closely related to baker's yeast. Even in this case, sequence homology is essentially restricted to a region of 100 amino acid residues corresponding to the DNA-binding

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   1  GAG AGA GAG AAA ACA AAT CAA GTT TTT TTA GAG AAA GAA AAG ATA GTT ATG GTC GTG TTG TTG TTG GTG AAG ATT GGC AAA GTT      84

  85  GTA GCC AAT GGA AGA TTC TAA GAT TAT CAT CAA TCG TCC TAA AAA TAA TCT TGT TCA TTC TCT AGT CTT TCT TTT CGG TTT CTA     168

 169  TTG TTG GGA TTG GGA ATT TCT TAC TGC CCT TTT GCT TTT CAG TTA TTG CTC CTT CTA ATT AAT TTC TGT AAA ATT GTA AAA TTC     252

   1                                                                                                  Met Ser Gln       3
 253  TTA TAT AGG ATC TTG TCA ATT AAT AAG TAA AAT CCC CAA AAG GGG AAA TTT GGG TAG GTT TAG AGA AAG AGG GGG ATG TCG CAA     336

   4  Arg Thr Ala Pro Ala Pro Phe Leu Leu Lys Thr Tyr Gln Leu Val Asp Asp Ala Ala Thr Asp Asp Val Ile Ser Trp Asn Glu      31
 337  AGA ACA GCG CCG GCG CCG TTT CTG TTG AAA ACG TAT CAG TTG GTG GAT GAT GCC GCC ACT GAC GAT GTG ATA TCT TGG AAT GAA     420

  32  Ile Gly Thr Thr Phe Val Val Trp Lys Thr Ala Glu Phe Ala Lys Asp Leu Leu Pro Lys Tyr Phe Lys His Asn Asn Phe Ser      59
 421  ATC GGC ACA ACC TTT GTG GTG TGG AAA ACT GCT GAA TTT GCA AAG GAT TTG CTT CCC AAA TAC TTC AAG CAC AAT AAT TTC TCC     504

  60  Ser Phe Val Arg Gln Leu Asn Thr Tyr Gly Phe Arg Lys Ile Val Pro Asp Lys Trp Glu Phe Ala Asn Glu Asn Phe Lys Arg      87
 505  AGC TTC GTT CGA CAG CTT AAC ACC TAT GGT TTT CGA AAG ATT GTG CCT GAC AAA TGG GAA TTC GCC AAT GAG AAC TTC AAA AGA     588

  88  Gly Gln Lys Glu Leu Leu Thr Ala Ile Arg Arg Arg Lys Thr Val Thr Ser Thr Pro Ala Gly Gly Lys Ser Val Ala Ala Gly     115
 589  GGA CAG AAA GAG CTC CTC ACA GCA ATA CGC CGT CGG AAG ACC GTG ACA TCA ACC CCA GCT GGT GGA AAG TCT GTG GCA GCT GGG     672

 116  Ala Ser Ala Ser Pro Asp Asn Ser Gly Asp Asp Ile Gly Ser Ser Ser Thr Ser Ser Pro Asp Ser Lys Asn Pro Gly Ser Val     143
 673  GCT TCA GCA TCT CCG GAC AAT TCG GGG GAT GAC ATA GGT TCA AGT TCT ACC TCG TCC CCA GAC TCC AAG AAC CCG GGA TCT GTG     756

 144  Asp Thr Pro Gly Lys Leu Ser Gln Phe Thr Asp Leu Ser Asp Glu Asn Glu Lys Leu Lys Lys Asp Asn Gln Met Leu Ser Ser     171
 757  GAC ACT CCG GGA AAG TTG TCC CAG TTC ACG GAC TTG TCG GAT GAG AAT GAG AAG CTA AAG AAA GAC AAC CAG ATG CTG AGC TCA     840

 172  Glu Leu Val Gln Ala Lys Lys Gln Cys Asn Glu Leu Val Ala Phe Leu Ser Gln Tyr Val Lys Val Ala Pro Asp Met Ile Asn     199
 841  GAG CTG GTG CAG GCG AAG AAA CAA TGC AAT GAG TTG GTT GCT TTC TTG AGC CAG TAC GTG AAG GTT GCA CCG GAT ATG ATC AAC     924

 200  Arg Ile Met Ser Gln Gly Thr Pro Ser Gly Ser Ser Leu Glu Glu Leu Val Lys Glu Val Gly Gly Val Lys Asp Leu Glu Glu     227
 925  CGT ATC ATG AGC CAA GGA ACC CCA TCG GGG TCC AGC CTT GAA GAG TTG GTC AAG GAG GTT GGT GGT GTT AAA GAT TTA GAA GAA    1008

 228  Gln Gly Ser Tyr Asn Asp Asn Asp Asp Lys Glu Asp Asp Asp Glu Lys Gly Asp Thr Leu Lys Leu Phe Gly Val Leu Leu Lys     255
1009  CAG GGT AGT TAT AAT GAC AAC GAT GAT AAA GAA GAC GAT GAC GAA AAG GGT GAC ACT CTG AAA CTA TTT GGT GTG TTG CTG AAA    1092

 256  Glu Lys Lys Lys Lys Arg Gly Pro Asp Glu Asn Ile Glu Thr Cys Gly Gly Arg Gly Lys Met Met Lys Thr Val Asp Tyr Asn     283
1093  GAA AAG AAG AAG AAA AGG GGC CCG GAT GAG AAT ATT GAG ACT TGT GGT GGA CGT GGT AAA ATG ATG AAA ACT GTG GAC TAT AAT    1176

 284  Gly Pro Trp Met Lys Met Ser Ser Pro Ala Gly Glu Ser Ser Lys Val Cys Asn ***                                        302
1177  GGT CCT TGG ATG AAA ATG TCT TCG CCG GCC GGA GAA AGC AGC AAG GTT TGT AAC TGA GGT GAA GTT GGT GAA CTA TGT GTT GAG    1260

1261  GTT GGA GCA GAG TAA ATT AGC TTC TCC CTT TTG ATT TAG TAA TTT CCT TTG GAT TTA CAA GTT CGA TGT TTA TGA ATA AAA TAA    1344

1345  ATA GAG AAT TTA ATT AGT ATG AGT AGT AAA AAG GGT TTA GAT TAC TAC TAA GAG TAG TAC ACC CAA CTA GAA ATT ATA TAT ATA    1428

1429  TAC ATG CAG ACT GCT ATC AAG TCA AAT GCA ACT CAA GTT TGC TTT GTC TAG TAA GGA ACA TAA TAA TAA TAA TAT TGG TTC TTG    1512

1513  ATA AAA AAA AAA AAA AAA                                                                                        1530
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Fig. 5. Nucleotide sequence of the cDNA clone HSF24. The nucleotide sequence derived from the cDNA starts in the 5′ leader region and terminates in a poly(A) containing sequence downstream from a stop codon. The open reading frame comprises 301 amino acid residues. The putative HSE-binding domain is boxed. Circles mark a regular pattern of hydrophobic amino acid residues (potential Leu-zipper), and three prominent proline residues are highlighted by open arrowheads.

domain of the yeast HSF (B.Jakobsen and H.R.B.Pelham, cited in Sorger and Nelson, 1989).

Though functional analyses are just in progress, we want to point at two structural elements, which may be significant for the interaction with hs promoters. First, the binding domain is flanked by proline residues (Figure 5), and second, it contains two overlapping repeats of about 40 amino acid residues, each with a proline residue in its center (see also arrows in Figure 6B):

$$(24)\quad W(7X)\,{}^{V}_{I}(7X)\,{}^{A}_{V}-(4X)-P\,X\,{}^{Y}_{F}(5X)\,F(6X)\,L(5X)\,{}^{R}_{H}K\quad(67)$$

$$(53)\quad F(6X)\,L(7X)\,{}^{V}_{I}X\,P(2X)W\,X\,F(4X)\,F(6X)\,L(5X)\,R\,{}^{R}_{Q}\quad(95)$$

Recently, another yeast gene (flocculation suppressor SFL1), seemingly unrelated to the hs response, was sequenced and shown to exhibit a remarkable homology with the N-terminal two-thirds of the HSE-binding domain (Fujita *et al.*, 1989). The relevance of this result, e.g. with respect to its DNA-binding specificity, remains to be analyzed.

A coexistence of several different HSE-specific DNA binding proteins has not yet been demonstrated for any other organism. But this may simply reflect differences between the screening methods applied, i.e. South-Western screening (this paper) versus immune screening (Sorger and Pelham, 1988; Wiederrecht *et al.*, 1988). It is tempting to speculate that the hs response initially involves activation of a constitutive factor such as HSF8 to bind to HSE as shown by the gel retardation assay (Figure 1). Under continued stress conditions newly formed factors, e.g. HSF24 and HSF30, may be required to assist or respectively replace HSF8 in its activity to facilitate hs gene transcription. A homooligomeric form of HSF8 may be replaced by heterooligomeric forms involving HSF8, 24 and/or 30. Crosslinking of the DNP complexes and careful footprint experiments with different hs promoters will be required to analyze the supposed fine tuning of hs transcription. It may be that changes in the composition of the 'heat stress transcriptosome' by interaction with different HSF components are involved in the well known differential response of individual hs genes with respect to the types of inducer, to the temperature threshold and the time course of expression (Nover and Scharf, 1984; see summary in Nover, 1990).

Multiple HSFs are reminiscent of the situation in *E.coli*, where two hs sigma factors are required, σ^{32} for the transient activation and σ^{24} for permanent activation under severe hs conditions (Nagai *et al.*, 1990; Straus *et al.*, 1989; Wang and Kaguni, 1989). But several reports document unexplained peculiarities of hs regulation in animal systems as well, which may indicate the existence of more than one HSF (Johnston and Kucey, 1988; Metzger *et al.*, 1989; Mitani *et al.*, 1990; Zimarino *et al.*, 1990).

It was pointed out earlier that the successful application of the DNA-ligand screening depends on the fidelity of binding activity of the HSF domains inserted at the C-terminus of a β-galactosidase fusion protein (Figures 2 and 3). In contrast to the native HSF, hs activation of the binding was not required in these cases. This may reflect an artificial exposure of the DNA-binding domain due to

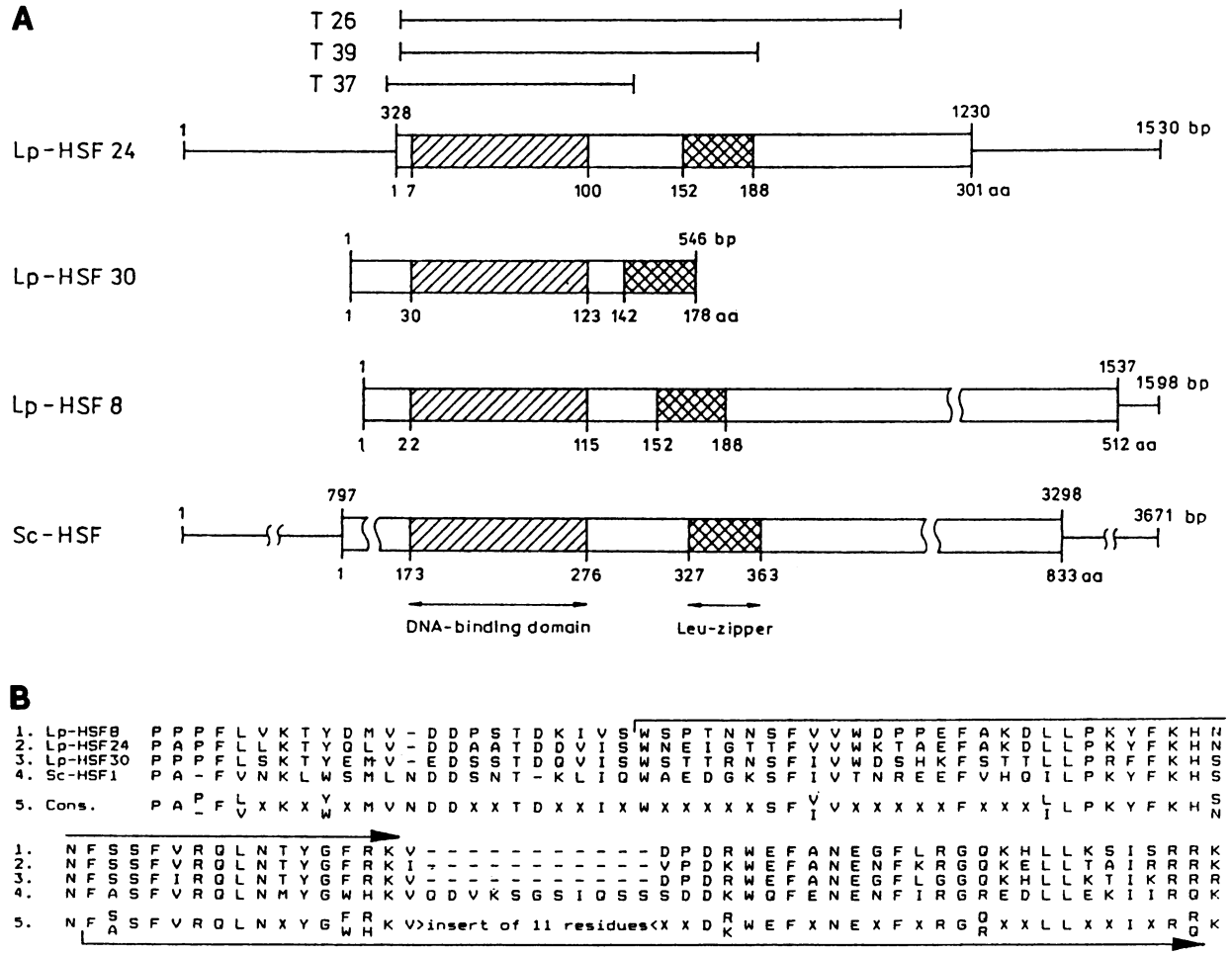

Fig. 6. Comparison of the putative HSFs of tomato (Lp-HSF) with the yeast HSF. **A**, Sequence organization: hatched bars, potential HSE-binding domains; cross-hatched bars, sequences with a regular pattern of hydrophobic amino acid residues; open bars, regions without evident sequence homology. Figures above the line indicate the number of nucleotides, figures below refer to amino acid residues. The length and alignment of the three partial clones of HSF24 are indicated by the lines on top. **B**, Amino acid sequence of the potential HSE-binding domains: The three partial sequences of the tomato clones are compared with that of the bakers yeast (Sc-HSF1, Sorger and Pelham, 1988; Wiederrecht *et al.*, 1988). The consensus sequence (5) denotes invariable amino acid residues. The long overlapping repeats mentioned in the text are marked by arrows.

Table I. Molecular characteristics of the three HSF clones of tomato

Clones	No. of related clones isolated[a]		Insert size (kb)	Oligonucleotide binding[b,c]			Messenger RNA		
							Size	Abundance[c]	
	C	HS		HSB_n	HSB	HSB_nmt	(kb)	C	HS
Lp-HSF8	1	–	1.5	+	+	–	2.0	++	++
Lp-HSF24	–	9	0.38–0.85	++	+(+)	–	1.5	++	++++
Lp-HSF30	–	1	0.58	+++	+++	–	1.4	(+)	++++
							1.9	–	++

[a]Clones were isolated by South-Western screening of cDNA libraries prepared from control (C) and heat shock (HS) mRNAs.
[b]Details of the HSB monomer, oligomer (HSB_n) and the mutant HSB_n are described in Materials and methods.
[c]+, ++, +++, and – indicate estimates of binding activities and mRNA concentrations respectively.

the N-terminal sequence of the β-gal-protein. On the other hand, the β-gal part of this chimeric protein may be essential for oligomerization in *E.coli*. Trimerization of the yeast and *Drosophila* HSF subunits was shown to be a prerequisite for efficient HSE—binding (Perisic *et al.*, 1989; Sorger and Nelson, 1989). At any rate, HSE binding and activation of transcription in the multicomponent transcriptosome complex may be independent events of the signal transduction (Hensold *et al.*, 1990; Jakobsen and Pelham, 1988; Larson *et al.*, 1988; Metzger *et al.*, 1989; Mosser *et al.*, 1988).

Materials and methods

Oligonucleotides and labelling

The following oligonucleotides were kindly provided by Dr Herrmann (Central Institute of Molecular Biology, Berlin–Buch)

HSB:
5'-tcgaggatcctaGAAgcTTCcaGAAgcTTCtaGAAgcagatc -3'
3'- cctaggatCTTcgAAGgtCTTcgAAGatCTTcgtctagagct-5'
HSB, mutant:
5'-tcgaggatcctatAAgcTTacatAAgcTTatatAAgcagatc -3'
3'- cctaggataTTcgAAtgtaTTcgAAtataTTcgtctagagct-5'

Oligonucleotides were labelled with [$\gamma-^{32}$P]ATP using T4 kinase, followed by annealing and ligation in the presence of T4 ligase (Maniatis *et al.*, 1982). For the primary screening the HSB was ligated overnight at 4°C in the presence of T4 ligase to give a mixture of oligomers (HSB_n with n=1−6) (Kadonaga *et al.*, 1986; Vinson *et al.*, 1988). For the gel retardation assay the two HSB oligonucleotides were annealed and labelled by filling in of the overhanging ends with Klenow polymerase (Maniatis *et al.*, 1982).

cDNA library and South-Western screening

cDNA was synthesized from poly(A)$^+$RNA of control and heat-stressed tomato cell suspension cultures (Nover *et al.*, 1982; Nover and Scharf, 1984) and ligated into λgt11 using the λ librarian cloning system of Invitrogen (San Diego, USA). Packaging was done with the Gigapack Plus System of Stratagene (La Jolla, USA).

South-Western screening of the random-primed cDNA and preparation of recombinant λ phages from *E.coli* Y1090 followed the procedure of Singh *et al.*, 1988, 1989). The fidelity of binding of the lacZ fusion proteins to the HSB oligonucleotide was monitored by comparing the signals obtained with the oligomerized HSB_n, with the monomer and with the oligomerized mutant HSB respectively (Figure 2). The HSF24 clone was isolated by plaque hybridization-screening of an oligo(dT)-primed λgt11 library using the T37 partial clone as probe.

Gel retardation assay

The origin and cultivation of *Lycopersicon peruvianum* (tomato) suspension cultures have been described previously (Nover *et al.*, 1982). For preparation of crude nuclear fractions (Nover *et al.*, 1986) 25 ml of tomato suspension culture were sucked off and the cells (~2 g wet weight) were homogenized in 5 ml low salt buffer H (50 mM Tris−HCl, pH 7.5, 25 mM NaCl, 5 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM EDTA, 0.1% Nonidet P40, 14 mM β-mercaptoethanol, 5% sucrose, 30% glycerol). After resedimentation, the crude nuclear pellet was extracted with high salt buffer E (20 mM Tris−HCl, pH 8.0, 400 mM NaCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 5% glycerol). After 30 min centrifugation at 4°C (15 000 r.p.m., Eppendorf rotor) the supernatant was frozen in liquid nitrogen. For protection against proteases both buffers contained 3.5 μg/ml aprotinin and 1 μg/ml leupeptin.

The binding assay and electrophoresis was adapted from Revzin (1989), Sorger *et al.* (1987) and Zimarino and Wu (1987). Usually, 800 ng salmon sperm DNA or poly(dI·dC) and 1 ng ^{32}P-labelled HSB in 10 μl binding buffer B (20 mM Tris−HCl, pH 8.0, 50 mM NaCl, 15 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT, 10% glycerol) were incubated with 5 μl nuclear extract containing 10 μg protein. After 10 min at 20°C the incubation mixture was separated on a 5% polyacrylamide gel with 0.5×TBE/20% glycerol as gel buffer and 0.5×TBE as running buffer (Maniatis *et al.*, 1982). Essential for the quality of the retardation assay are the 15 mM $MgCl_2$ in buffer B, the glycerol content of buffers B and TBE and a sample gel (2.5% polyacrylamide/0.5% agarose) on top of the separation gel.

For the retardation assay with the lacZ fusion proteins the recombinant clones were propagated as phage inserts of lysogenic *E.coli* strain Y1089. After induction with IPTG bacteria were harvested, frozen in 200 μl buffer E containing 100 mM NaCl and afterwards disrupted by sonication. After centrifugation the extract was stored in liquid nitrogen and used for the retardation assay as indicated above.

Other methods

cDNA inserts subcloned into pBSK+ BluescriptII vector (Stratagene, La Jolla) were sequenced by the dideoxy chain termination method of Sanger *et al.* (1977) using the T3 and KS primers. Southern and Northern blot hybridizations followed standard procedures (Maniatis *et al.*, 1982).

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Note added in proof

The biological significance of the HSF8 and HSF24 clones is supported by hs-dependent transactivation of a hs promoter–gus reporter construct in a transient expression assay using tobacco protoplasts (E.Treuter *et al.*, in preparation).