Chapter 14

The Expression of Heat Shock Genes — A Model for Environmental Stress Response

Fritz Schöffl, Götz Baumann, and Eberhard Raschke

Universität Bielefeld, Fakultät für Biologie, Genetik, D-4800 Bielefeld 1, Federal Republic of Germany

With 4 Figures

Contents

I. Introduction

A. The Heat Shock Response

When cells, tissues, or whole organisms are subjected to grossly elevated temperatures or heat shock (hs), they respond transiently by synthesizing a number of new proteins, the prevalent heat shock proteins (hsps). Hsps are operationally defined as those proteins whose synthesis is immediately and dramatically induced at high temperatures. The induction of hsp synthesis is the major feature of the hs response; other aspects of the reprogramming of cellular activities are less well understood (for recent reviews see Burdon, 1986; Lindquist, 1986; Nagao *et al.*, 1986; Schlesinger, 1986; Schöffl *et al.*, 1986). Some of the general characteristics relevant to plants are briefly summarized here.

In plants, both an abrupt shift and a gradual increase of temperature seem to effectively elicit the hs response. The maximum response is usually

D. P. S. Verma et al. (eds.), *Temporal and Spatial Regulation of Plant Genes*

achieved at temperatures 10—15°C above optimum growth temperature, correlating with the range of exposure under natural conditions. The induction of hsp synthesis is very rapid with a maximum activity within 1—2 hours and a reduced rate continuing for several hours. Sustained synthesis may only be observed when the severity of the hyperthermic stress is increased and cells begin to die. The threshold temperatures sublethal to plants may also vary depending on the normal range of environmental growth conditions, the duration of heat stress, and eventually on a conditioning pretreatment at high temperatures. These temperatures range from 37°C to about 45°C for most plants and several hours of treatment; however, desert succulents (Cacti and Agaves) may survive much higher temperatures for extended periods of time (Kee and Nobel, 1986).

Short pulses of extreme and otherwise lethal hyperthermia induce tolerance to these temperatures for longer periods, provided the shocks are intermitted by a sufficiently long recovery period (usually more than two hours) at lower temperatures (Lin *et al.*, 1984). The acquired thermotolerance in most cases protects plants in the natural environment from seasonal and diurnal hot spells. Plants in the field respond to the natural changes in ambient temperature in summer in a similar way as to hs simulation in the laboratory; however, only when the water supply is limited (Kimpel and Key, 1985; Burke *et al.*, 1985). Leaf temperatures of irrigated plants are usually below the threshold temperatures required for the elicitation of the hs response, probably due to the cooling effect of stomatal transpiration.

B. Groups of Related Heat Shock Proteins

The hsps in plants can be divided into several groups based on molecular masses, homologies of derived amino acid sequences, and subcellular compartmentation:

(i) High molecular mass (hmm) hsps ranging from 80 to 100 kDa. Two different hsps from this group usually occur in any given plant species. Genes with homologies to the *Drosophila* hsp83 have been recently isolated from soybean (Roberts and Key, 1985), *Brassica oleracea* (Kalish *et al.*, 1986), *Arabidopsis thaliana,* and *Zea mays* (Dietrich *et al.*, 1986). These proteins are probably also related to the other hsp83-homologous proteins from chicken (hsp90 and hsp108) and yeast (hsp90) (Blackman and Meselson, 1986; Kleinsek *et al.*, 1986; Sargan *et al.*, 1986) and with the hmm-hsps in human, mouse, and frog (Schlesinger *et al.*, 1982). The most interesting property of proteins in this group may be their capability to reversibly bind other proteins in the cytoplasm as shown by the interaction of hsp89 with pp50 and $pp60^{src}$ (Brugge *et al.*, 1981; Oppermann *et al.*, 1981), hsp90 with the 8S progesterone receptor (Catelli *et al.*, 1985) and hsp90 and hsp100 with polymerizing actin from rabbit skeletal muscle (Koyasu *et al.*, 1986), suggesting that these hsps may serve to carry important proteins around the cell in their inactive form.

(ii) The hsp70 family with molecular masses ranging from 68 to 70 kDa in different plants. Hsp70 is perhaps the most conserved gene product in nature as indicated by the 73% homology between human and *Drosophila* and 50% homology between the fly hsp70 and the *E. coli dna*K protein. Maize hsp70 genes share regional homology (75% in the N-terminal part of the protein coding region) with their counter part in *Drosophila* (Rochester *et al.*, 1986) which is also homologous to hsp70 genes in soybean (Roberts and Key, 1985), and *Arabidopsis thaliana* (Coldrion-Raichlen *et al.*, 1986).

Molecular models for the function of hsp70 are based on the capacity of the protein to bind and hydrolyze ATP, its reversible interaction with other proteins, and its localization in the nucleus/nucleolus during heat shock (Pelham, 1986). It is postulated that hsp70 has a general affinity for denatured or abnormal proteins, probably by hydrophobic interaction. This property may limit the formation of insoluble aggregates and protect proteins from proteolytic degradation.

(iii) Small or so-called low molecular mass (lmm) hsps, 15—20 kDa, are particularly prominent and heterogenous in plants (for review see Schöffl *et al.*, 1986). Sequence homology between lmm-hsps of different plants was shown by the cross hybridization of soybean cDNA probes with hs-induced mRNAs from pea, sunflower, millet, corn, parsley, and broad bean. These proteins tend to form higher order structures (cytoplasmic granules in tomato and soybean) or become associated with nuclei and other subcellular fractions containing organelles. Similar properties are also described for the *Drosophila* lmm-hsps (hsp22, -24, -26, -27). Features of the polypeptide structure common to plant and animal hsps are discussed later.

Other small hsps with molecular masses between 21 and approximately 30 kDa are less abundant in soybean, pea, maize, and other plants. Some of these proteins are transported into chloroplasts and mitochondria; two polypeptides, 21 and 24 kDa, become stably associated with mitochondria in soybean seedlings during heat shock and recovery (Lin *et al.*, 1984). Other precursor hsps with molecular masses between 27 kDa and 30 kDa are translocated into chloroplasts of pea, soybean, maize, and *Petunia* (Kloppstech *et al.*, 1985; Vierling *et al.*, 1986; Vierling *et al.*, 1987 a); the molecular masses of the mature proteins within the chloroplasts are 22 kDa in pea, 20—22 kDa in soybean, and 20—23 kDa in *Petunia*. The biological function of these proteins is still obscure but they appear to be structurally related to the other small cytoplasmic hsps and they may be involved in similar processes inside and outside the chloroplast (Vierling *et al.*, 1987 b). Efficient *in vitro* import of a cytosolic soybean hsp into pea chloroplasts occurred only when the protein (Gmhsp17.5-E) was genetically fused to the transit peptide of the small subunit of ribulose 1,5-bisphosphate carboxylase (Lubben and Keegstra, 1986). Although the imported hsp appeared to be targeted to the stroma, this may not be its final destination during heat shock. *In vitro* translocated native pea hsp22 is also targeted to the stroma in chloroplasts from normal plants, but it becomes associated with thy-

lakoid membranes in chloroplasts from heat-stressed tissues (Glaczinski and Kloppstech, 1987). Although all the hsps described so far are encoded in the nucleus, there is evidence that *Brassica* and maize hs-induced proteins (62 kDa and 60 kDa) may be encoded in the mitochondrial genome (Sinibaldi and Turpen, 1985).

C. Heat Shock and Other Environmental Stresses

When plants are subjected to adverse environmental conditions other than heat shock, their cells respond to these stresses by changes in gene expression in a stress-specific manner (for a review see Sachs and Ho, 1986). However, certain stressors induce also some of the hsps in different plant species. This observation evokes two important questions:

(i) Is there a common molecular mechanism of hsp synthesis induction (gene activation) provoked by different environmental stresses?

(ii) Is there a functional interrelationship among the common hsps induced by the different stresses?

Several hypotheses have been put forward for the signal transduction induced by heat shock and a bewildering variety of stress agents in animal cells and bacteria (for a review see Nover *et al.*, 1984). One theory was based on the coinduction of hsps and unusual bisnucleotides, termed "alarmones", by many stressors; therefore it was proposed that alarmones trigger the hs response. This hypothesis has been discredited recently by srutinizing the effect of hyperthermia on hsp synthesis and alarmone concentration in *Xenopus* oocytes and cultured hepatoma cells (Guedon *et al.*, 1986). It was shown that hsps are already induced under conditions which do not significantly change the level of alarmones.

Mitochondria were also implicated in playing an important role in the hs response, since many other physico-chemical stresses, most of which interfere with oxidative phosphorylation of electron transport, induce hsp synthesis in *Drosophila.* Although anaerobic stress or recovery from anaerobiosis also induces hsps in many organisms, anaerobiosis in plants induces a different set of proteins (for a review see Sachs and Ho, 1986). Hsp synthesis, oxidative stress, and alarmones are not obviously linked in plants. Only arsenite and cadmium, two out of 20 tested physical and chemical stresses, induce mRNA and hsp synthesis in the soybean hypocotyl (Czarnecka *et al.*, 1984; Lin *et al.*, 1984). It seems conceivable that abnormal and denatured proteins which accumulate in the cells during stresses serve as stress signals and trigger the activation of hs genes. One model proposes that the interaction of ubiquitin with the proteolytic degradation system of the cell leads somehow to the activation of the hs transcription factor, HSTF (Munro and Pelham, 1985; Anathan *et al.*, 1986). HSTF subsequently recognizes and binds to hs promoter elements (HSE) upstream from hs genes (for a review see Pelham, 1985). According to this model, heat shock and arsenite trigger the hs response in plants in a very

similar way. Heat-inducible transcription of soybean hs genes was demonstrated by run-off transcription in isolated nuclei (Schöffl *et al.*, 1987), and the DNA sequence analysis revealed multiple copies of HSE-like sequences in the upstream promoter regions (discussed later). Cadmium may possibly activate hs genes via a different transcription factor that also interacts with other promoter binding sites. Such conserved binding sites, termed metalresponsive elements or MRE, were identified in the promoter of metallothionine genes (Karin *et al.*, 1984), but homologous sequences are also present in the upstream regions of plant hs genes (Czarnecka *et al.*, 1985). The activation of certain human hsp70 genes by different stresses seems to require different promoter elements, e. g. for serum stimulation and cadmium induction (Wu and Morimoto, 1985). The developmental induction of the *Drosophila* hsp26, -28, and -83 genes seems to be regulated by another independent form of interaction between promoter element and protein (Hoffman *et al.*, 1987; Mestril *et al.*, 1986).

In plants, cell-specific differences and developmental induction of hsps have not yet been critically examined. Different tissues of a plant species usually synthesize identical sets of hsps with the exception of pollen tubes and grains (Xiao and Mascarenhas, 1985; Zhang *et al.*, 1984; Cooper *et al.*, 1984). The inability to induce a chimeric hs-*npt*II gene in pollen from transgenic tobacco (Spena and Schell, 1987) and the generally lower thermotolerance of pollen is in accordance with the view that the ability to synthesize hsps is crucial to the development of thermotolerance. Several lines of evidence support this hypothesis:

(i) There is a tight correlation between the kinetics of hsp synthesis and the development of thermotolerance in many organisms.

(ii) Conditioning heat treatment of the soybean hypocotyl can be replaced by incubation at normal temperature in the presence of arsenite, a chemical inducer of hsp synthesis (Lin *et al.*, 1984). The overlap in the stress protein patterns (analysed by 2-D PAGE) induced by arsenite and heat shock is incomplete; but, remarkably, it includes the abundant lmm-hsps. Ethanol, hypoxia, and other agents affect some animal cell lines in a very similar manner and conversely, heat shock induces tolerance to these other physical and chemical stresses (see Lindquist, 1986 and refs. therein).

(iii) Eukaryotic cell lines selected for increased survival at high temperatures constitutively produce hsps, whereas mutants of *E. coli* and *Dictyostelium* which are unable to acquire thermotolerance are defective in hsp synthesis (see Lindquist, 1986 and refs. therein). Varietal differences between high-temperature-tolerant and susceptible lines of *Sorghum* correlate with temporal differences in the capacity to synthesize hsps during early germination (Ougham and Stoddart, 1986).

Despite overwhelming correlative evidence, exceptions seem to contradict a protective role for hsps and their causal connection to thermotolerance. For example, germinating pollen of *Tradescantia* which fail to synthesize hsps nevertheless acquire a certain level of thermal tolerance by

a conditioning heat treatment (Xiao and Mascarenhas, 1985). It seems conceivable that cells are able to cope with high temperature stress by different but concerting processes. Hsp synthesis may be crucial, but changes in lipid composition in membranes may also be important in plants (Bery and Björkman, 1980; Süss and Yordanov, 1986). Conversely hsps seem to protect the cells from harmful effects induced by other environmental stresses discussed above. The molecular mechanisms of such processes are not yet understood and it is unknown whether hsps act via the same mechanism during hyperthermia and chemical stresses.

Heat shock gene activation may be a good model for other environmental gene expression. The present data suggest common principles of induction for hs genes and in a broader sense also for the regulation of gene expression induced by other environmental stresses unrelated to heat stress and hs genes (for a review see Sachs and Ho, 1986). Investigations of these stress responses have received increased attention for scientific and agricultural reasons, but the preference of hs response as a model system is based on the accessibility (abundance of mRNAs and hsps) of the molecular processes of transcription, translation, etc., the rapidity of induction (within minutes following temperature rises), the coordinated expression of homologous genes (related gene families), and the advanced state of the molecular analysis of hs genes in both native and transgenic plants.

Soybean was the first plant used for these investigations and most molecular studies were performed on hs genes of this organism. Here we concentrate on this system with emphasis on the lmm-hsps, which are of paramount interest for plant-specific aspects of the hs response. The recent investigations of hsp70 genes in other plants are also discussed briefly.

II. Molecular Biology of Heat Shock Genes

A. Sequence Homology Among Small Heat Shock Proteins

Current knowledge of the amino acid sequences of hsps and their deduced secondary structures is based exclusively on DNA sequencing. Several cDNA and genomic clones for lmm-hsps (17—18 kDa) have been sequenced to date; six of the seven genes belong to the same gene family, class I, and one is a member of another family, class VI (see Table 1). The sequence homology within class I is about 90%, and the same degree of homology exists in class VI between the gene Gmhsp17.9-D (Raschke *et al.*, 1988) and a full-length cDNA clone pEC75 (Czarnecka *et al.*, 1984; Key *et al.*, 1985). The largest open reading frames encode polypeptides with calculated molecular masses of between 17.3 kDa and 18.5 kDa, assuming translation starts at the first ATG codon downstream from the 5′-terminus of the mRNA. The molecular masses of polypeptides translated *in vitro* from synthetic, SP6-polymerase-generated RNAs of Gmhsp17.3-B and Gmhsp17.6-L genes are consistent with the proposed

translational start sites (F. Schöffl and S. Krusekopf, unpublished results). The 5′ untranslated leader sequences are remarkably divergent and unusually long (75—104 bp) in proportion to their respective coding sequences (459—483 bp). The leader sequences are probably involved in the selective translation during heat shock as has been reported for *Drosophila* hsp22 and hsp70 (Hultmark *et al.*, 1986; McGarry and Lindquist, 1985; Klemenz *et al.*, 1985), but except for being A-rich (40%) there are no striking homologies between 5′ sequences of plant and animal hs-mRNA.

Table 1. Small Soybean Heat Shock Genes Characterized by DNA Sequencing

hsp-gene	synonymous designation [1])	length of polypeptide (amino acid residues)	length of 5' nontranslated leadersequences (bp)	References
class I				
Gm hsp 17.3-B	hs 6871	153	104±2	Schöffl et al. 1984
Gm hsp 17.5-M	-	153	88,93	Nagao et al. 1985
Gm hsp 17.6-L	-	154	93,96	Nagao et al. 1985
Gm hsp 17.5-E	-	154	82	Czarnecka et al. 1985
Gm hsp 18.5-C	hs 53	161	76±2	Raschke et al. 1987
class VI				
Gm hsp 17.9-D	-	159	72±2	Rascke et al. 1987

The DNA homology within the coding regions of soybean class I hs genes varies between 83—94% and 90—95% of the amino acid sequence (see Fig. 1). The average amino acid homology between class I and class VI

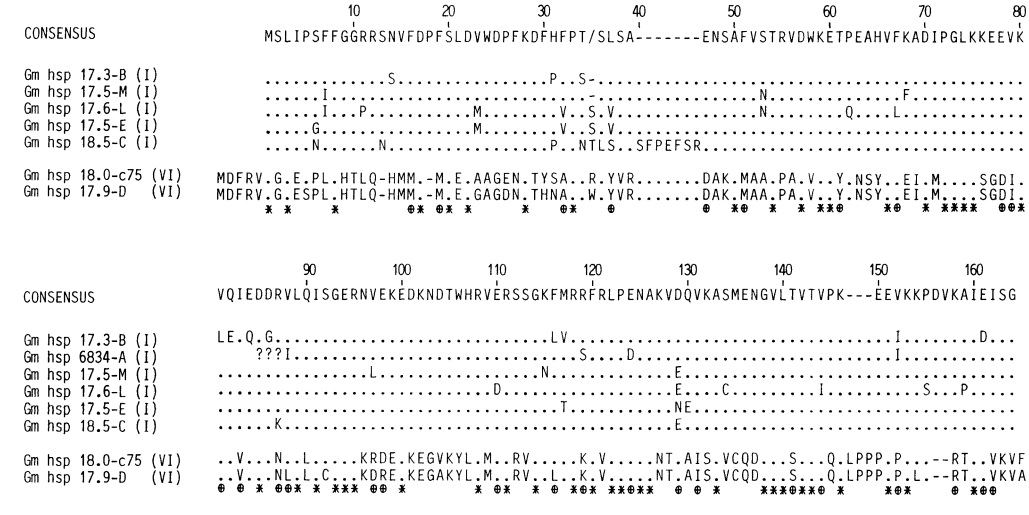

Fig. 1. Comparison of the amino acid sequences of small soybean hsp. +: Conservative amino acid substitutions, *: invariable amino acid positions (for origin of DNA sequence data see Table I)

hsps is only 38% and is more pronounced in the C-terminal portions of the polypeptides. Secondary structure predictions and the distribution of hydrophobic and hydrophilic domains reveal a high degree of conservation in this region (Raschke *et al.*, 1988; Schöffl *et al.*, 1986). The conserved structural features are:

(i) A hydrophilic domain at the amino acid positions 80—90 with the potential for α-helix formation, followed by (ii) a hydrophobic region (positions 130—135) with the almost invariant sequence GlyValLeuThrVal and a potential for β-sheet formation, and (iii) another hydrophilic and probably α-helical region towards the C-terminus formed from charged and polar amino acid residues.

These three domains may be the important determinants for the structure and function of the small hsps in plants. Similar domains occur also in related hsps in *Drosophila* (Southgate *et al.*, 1983; Ingolia and Craig, 1982; Ayme and Tissières, 1985), *Caenorhabditis* (Johnes *et al.*, 1986), humans (Hickey *et al.*, 1986) and in the α-crystallins of the vertebrate eye lens (de Jong, 1982; van den Heuvel *et al.*, 1985). It seems that the selection pressure conserving the secondary structure of small hsps nevertheless allows a considerable variation in DNA and amino acid sequences in different organisms. The small hsps from one species are highly homologous in their C-terminal halves (>90% within soybean class I and class VI, up to 75% in *Drosophila*), but homology is markedly lower for different hsp families in one species or between hsps in different species (Fig. 2). The two hsp families I and VI in soybean diverge in amino acid sequence from each other to the same degree as from the *Drosophila* small hsps. The structural differences between these soybean hsps seem to be insignificant and do not imply different functional properties of these proteins. It seems possible that these two gene families originated from structurally and functionally related ancestors by gene duplication or gene conversion at a time late in evolution. The numbers of hs genes within each family exceed the potential numbers calculated on the basis of ploidy level of present day *Glycine max* plants which have tetraploid genomes (Singh and Hymowitz, 1985; Lee and Verma, 1984). Thus, allelic variation cannot be the chief cause of the existence of gene families in soybean. Additional data are required to investigate the evolution of hs genes in plants. It is noteworthy that hs-induced mRNAs of other dicot plants cross hybridize with both class I and class VI cDNA probes (Key *et al.*, 1983; F. Schöffl, unpublished results).

The small hsps from *Drosophila* and *Caenorhabditis* share a higher degree of homology with vertebrate α-crystallin proteins than with each other. The occurrence of introns is an other possible criterion for establishing the family connection of small hsps in different organisms. Introns were found in *Caenorhabditis* and human but not in soybean and fly hsp genes. The presence of introns may possibly impair the expression of these genes under severe heat stress as shown for the hsp83 gene in *Drosophila*

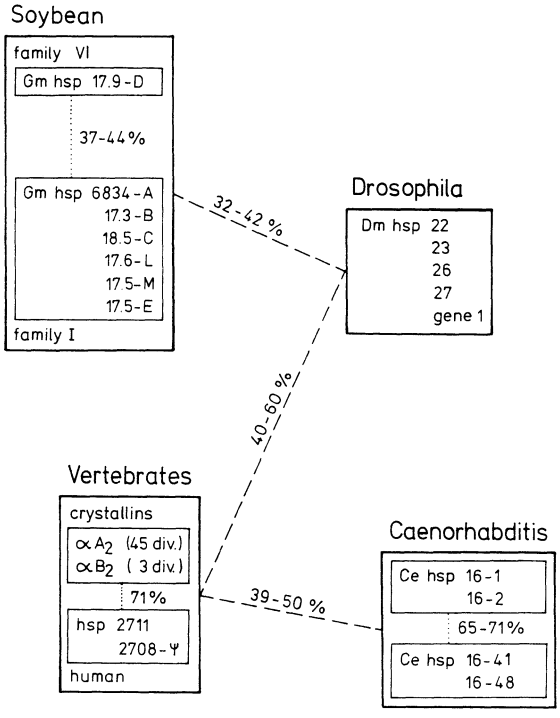

Fig. 2. Homologies among small hsps in and between different organisms. The homologies refer to regions encompassing the conserved domains in the C-terminal halves of the polypeptides, according to Raschke (1988)

(Yost and Lindquist, 1986). In contrast to those of other organisms, plant hsp70 genes seem to contain introns. Maize (Rochester *et al.*, 1986) and *Petunia* (J. Winter, personal communication) contain introns at the same position as the hsp70 cognate genes of *Drosophila* which are not expressed during heat shock.

B. Heat Shock Promoter and Upstream Sequences

The coordinate expression and transcriptional regulation of hs genes suggest a conservation of *cis*-active regulatory promoter and upstream sequences (Schöffl *et al.*, 1986, 1987). Dot matrix comparisons revealed local homologies in these regions between soybean hs genes of the two different gene families (Raschke *et al.*, 1988). Figure 3 shows an intergenic comparison with high resolution and stringency of the 5′-flanking regions of the genes listed in Table 1. Two patches of homologies appear in different areas of the plots. The proximal areas 5′ to the TATA boxes indicate homology of multiple HSE-like sequences. These palindromic sequence elements are clustered within about 150 bp upstream from the TATA box. A compilation of these sequences (Table 2) with 70% or more identity with the *Drosophila* consensus sequence 5′CT-GAA--TTC-AG

results in an extended palindrome with the consensus 5′TCTAGAA--TTCTAGA for soybean. Seven out of 10 symmetrical nucleotides in synthetic HSEs are sufficient for heat-inducible transcription in animal cells (Pelham and Bienz, 1982). The hs genes of all organisms analyzed so far contain HSE-like sequences starting 1.5 helical turns upstream from the TATA box in most genes (for a review see Bienz, 1985). Many of these genes contain multiple copies of HSE and several of them overlap with each other by four nucleotides (for soybean see Schöffl *et al.*, 1986; Raschke *et al.*, 1988). This configuration suggests phasing of HSEs by one helix turn or multiples of it (see Table 2). The significance of the spacing of HSE repetitions for transcriptional activation is not clear but it may reflect the potential for fine tuning of the regulation by direct and cooperative

Table 2. Compilation of Soybean HSE-like Sequences

Drosophila Consensus-HSE:		5' --CT-GAA--TTC-AG-- 3' (>> >>> <<< <<)	
Soybean	Pos.	⟶	Homology with Drosophila Consensus-HSE
Gm hsp 17.3-B	-276	ATCCCGAAACTTCTAGTT	9 / 10
	-245	GTCCAGAATGTTTCTGAA	7 / 10
	-235	TTTCTGAAAGTTTCAGAA	7 / 10
	-225	TTTCAGAAAATTCTAGTT	8 / 10
	-173	AACAAGGACTTTCTCGAA	7 / 10
	-163	TTCTCGAAAGTACTATAT	8 / 10
Gm hsp 18.5-C	-196	CTGTAGAAAGCTCTAGAA	8 / 10
	-186	CTCTAGAACTTGGGATTT	7 / 10
	-146	AAACAGAATTTTCTGGAA	7 / 10
	-136	TTCTGGAAAACACAGGAT	7 / 10
Gm hsp 17.9-D	-280	TTCTGGACATTACTAGAA	8 / 10
	-270	TACTAGAAAGATCCGAAG	7 / 10
	-221	TACTGGAAGTTTCACAGC	8 / 10
	-190	CTCCAGAAACTTCCATTT	8 / 10
	-146	CTTCAGAAACTTCCATTT	7 / 10
	-126	TTCTCGAATTATCTATGT	8 / 10
Gm hsp 17.6-L	-275	ATCTAGAAGGTTGTAGAA	9 / 10
	-253	AGCTAGAACGTACGTATT	7 / 10
	-224	GTCCTGAAGTTTATCGAA	7 / 10
	-214	TTATCGAATCATCTAAAA	7 / 10
	-155	TTCTGGAACATACAAGAG	9 / 10
Gm hsp 17.5-M	-600	ATCTTCAAACTTCAAGTT	9 / 10
	-430	AAAAACAATATTCTAGAA	7 / 10
	-359	CACAACAATATTTCAGAA	7 / 10
	-243	CACTAGAACCTTCGTACA	8 / 10
	-223	GAGTGGAGAAGTCCAGAA	7 / 10
	-213	GTCCAGAAGTTTTTATAG	7 / 10
	-160	AACACGATTTTTCTGGAA	7 / 10
	-150	TTCTGGAACGTACACGAT	8 / 10
Gm hsp 17.5-E	-535	TCCTCTATGGTTTCAGTG	7 / 10
	-513	GTTTGAAATTTTTTAGAT	7 / 10
	-495	TTCTTTAACATTCTAAAA	8 / 10
	-144	TTCTGGAACATACAAGAT	9 / 10
Soybean Consensus-HSE:		5' -TCTAGAA--TTCTAGA- 3' (>>>>>>> <<<<<<<)	

interaction with the transcription factor HSTF. The efficient targeting of HSTF to HSE may require a DNA distortion provoked by overlapping repeats and/or by the central purine and pyrimidine clusters in palindromes (Nover, 1987).

A second cluster of homologous sequences starts upstream from the HSE-containing region (#1 in Fig. 3). These signals correspond to short AT-rich repeats, starting with runs of "simple sequences" $(A)_n$, $(T)_n$, and $(AT)_n$. The repeated structure of the AT-rich intergenic sequences is most

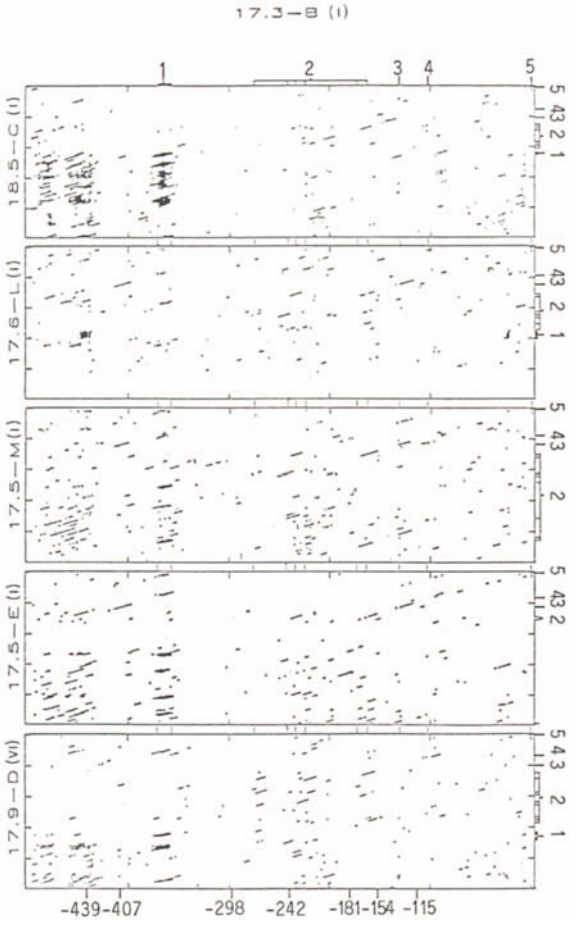

Fig. 3. Dot matrix analysis of the 5′ flanking sequences of soybean small hsp genes The analysis was performed for 500 nucleotides upstream from the translational start sites using a span of 15 with a match of 10 nucleotides (>67% homology). The numbers at the top and on the right-hand axes of panels refer to the positions of "simple sequences" (1), multiple HSE (2), TATA box (3), mRNA start (4), protein coding start (5). The numbers at the bottom panel refer to the endpoints of promoter deletions used for a functional analysis of Gmhsp17.3-B (see Fig. 4). For origin of DNA sequence data see Table 1

pronounced in the region upstream from Gmhsp 18.5-C, with two different consensus motifs, 5′TTTTTAA and 5′AAAAAT (Raschke *et al.*, 1988). Most other soybean hs genes are also proceded by blocks of AT-rich sequences. Emphasis is given to these simple sequences, which demarcate the upstream repeats (>75% A+T) and the proximal promoter regions (~55% A+T), because of their possible regulatory function in gene expression (see next chapter).

Dot matrix analyses also revealed the repetition of AT-rich motifs at positions downstream from the genes. Thus hs genes appear to be flanked by repetitive intergenic sequences. The full length of one repeat, upstream from Gmhsp17.9-D, is approximately 750 bp; those flanking the other genes were not completely sequenced. This organization of genomic sequences is in accordance with the short-period interspersion pattern (1.3 kb average unit length of single-copy DNA separated by 0.4 kb moderately repeated sequences) of the soybean genome (Gurley *et al.*, 1979). The flanking repeats may have structural and perhaps also regulatory importance, and they may have served as the targets for the processes leading to gene duplication.

C. Heterologous Expression of hs Genes in Transgenic Plants

The soybean lmm-hsp genes, Gmhsp17.3-B and Gmhsp17.5-E, were used to investigate hs gene expression in heterologous plant systems (Schöffl and Baumann, 1985; Gurley *et al.*, 1986). In these early studies, genes were introduced into sunflower using tumorigenic Ti-plasmid vectors. Thermoinduced synthesis of transcripts and the faithful initiation of the mRNAs demonstrated the functional integrity of plant hs promoters across phylogenetic barriers. The potential promoter sequences were delimited to approximately 1 kb (Gmhsp17.3-B) and 3.25 kb (Gmhsp17.5-E) upstream from the respective genes. A 5′-deletion to −1.175 kb resulted in a large increase in basal transcription, and a deletion to −95 bp curtailed hs-inducible RNA to 5% of the regular level of hs-induced transcription of Gmhsp17.5-E in primary tumors. These results rendered no definitive conclusions about the function of conserved promoter structures including the HSE-like sequences.

Sequences with homologies to the metal-responsive elements (MRE), the SV40 core enhancer sequence and other potential regulatory eukaryotic sequences, are not preferentially clustered in the plant hs gene promoters. MRE-like sequences upstream from Gmhsp17.5-E (Czarnecka *et al.*, 1985) may be important for the cadmium regulation of this gene (Gurley *et al.*, 1986), but a more critical analysis of this promoter will be required to discriminate between the two separate modes of induction via HSE and MRE respectively.

As a consequence of the known ambiguities of gene expression analyses in tumor tissues we changed the experimental strategy for our subsequent investigations of Gmhsp17.3-B. The gene was transferred into tobacco via leaf disk transformation with the binary T-DNA vector *Bin19*

(Schöffl *et al.*, 1986). Gene expression was studied in regenerated transgenic plants using Northern blot hybridization and S1-nuclease mapping of hs-induced transcripts. The analysis revealed equivalent levels of Gmhsp17.3-B mRNA in normal soybean and transgenic tobacco plants. The functional analysis of sequences required for transcriptional activation of this gene in tobacco was based on a series of 5′ deletions in the promoter region (Baumann *et al.*, 1987). For each deletion, seven or eight independently transformed plants were tested for the induction of mRNA synthesis by heat shock. Despite clonal variations of transcript levels in different plants, the average level of hs-induced mRNA declined in a nonlinear fashion with progressive deletions (Fig. 4). At least two different functions can be assigned to distinct parts of the hs promoter:

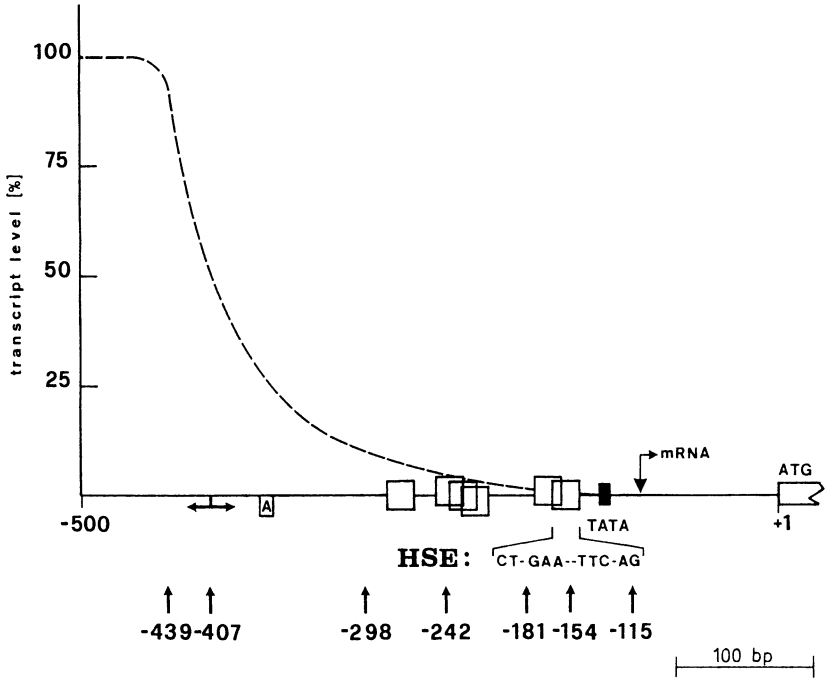

Fig. 4. Schematic diagram of the transcriptional activity of Gmhsp17.3-B upstream deletions in transgenic plants
The transcript levels obtained for the different deletions (vertical arrows) after heat shock in tobacco were determined by Northern hybridization using hs-induced mRNA in soybean as a standard (data from Baumann *et al.*, 1987). HSEs are marked by squares, A denotes a run of A ("simple sequences"), the bi-pointed horizontal arrow marks a hyphenated dyad

(i) Thermoinduced transcription requires the sequences between positions −181 and −154, a region containing the first two overlapping HSEs proximal to the TATA box. Additional HSEs between positions −298 and −181 seem to modulate moderately the level of transcripts.

(ii) The upstream sequences delimited by the deletions −439 and −298 lack HSEs; however, this region is required for maximal transcription. This stretch of DNA enhances the mRNA level by a factor of 10. The structural features within this region include an imperfect dyad symmetry encompassing 34 bp with its centre at position −407 and a run of adenine bases between the positions −357 and −371.

It is important to note that full promoter activity was achieved in plants transformed by the −439 deletion. Thus the essential *cis*-regulatory sequences are contained within 335 bp upstream from the start site of transcription (position −104). The bipartite structure of this plant hs gene promoter is quite unlike the known hs gene promoters in animals where transcriptional control seems to be solely based on HSE. The HSE-like elements are also essential for thermoinduced transcription in plants; enhancer-like sequences have not yet been discovered in the other hs genes. It is not known whether these characteristics of a hs gene promoter are typical for plant genes. The sparse data on the other hs genes in connection with inappropriate gene constructs and/or assay systems render it difficult to make a comparison. The conservation of the important structural features in most soybean hs gene promoter and upstream regions (see Fig. 3) and a growing body of information about enhancer based gene regulation in plants encourage us to predict the same principles for the other hs genes. In most other studies attention was given only to the presence of required HSE-like sequences, as for hs-induced transcription of Gmhsp17.5-E in sunflower tumors (Gurley *et al.*, 1986), Zmhsp70 in transgenic petunia (Rochester *et al.*, 1986), and a chimeric *npt*II gene controlled by the *Drosophila* hsp70 promoter in tobacco (Spena *et al.*, 1985; Spena and Schell, 1987). The combination of HSEs with upstream sequences from unrelated genes may generate plant promoters with novel functional properties. The fusion of an HSE-containing promoter fragment from soybean with the upstream region of a light-inducible gene from pea results in a light-dependent, heat inducibility of a linked reporter gene (Strittmatter and Chua, 1987). Bipartite promoter structures may also be an important feature of non-hs genes as indicated by the requirement of upstream sequences from constitutive genes for the anaerobic regulation of a maize *Adh*-1 promoter in tobacco (Ellis *et al.*, 1987).

In transgenic systems, except Gmhsp17.3-B gene in tobacco (Schöffl *et al.*, 1986; Baumann *et al.*, 1987), one could not achieve the same activity as the promoter in its native genetic background. This may be the result of the close proximity of promoter and enhancer sequences in Gmhsp17.3-B and the lack (or a larger distance from promoter) of enhancer-like sequences in the other constructs.

The upstream enhancer-like sequence shares no preferential homology with the SV40 enhancer core sequence or with any other known enhancer except for the dA : dT simple sequences that function as upstream enhancer elements in yeast (Struhl, 1985). Similar runs of simple sequences $(A)_{14}$, $(A)_{13}$, $(AT)_{15}$, and $(T)_{10}(A)_{12}$ occur within 350 bp upstream from the transcriptional start site of Gmhsp17.3-B, −18.5-C, −17.6-L and −17.9-D.

Soybean genes encoding the small subunit of ribulose bisphosphate carboxylase and leghemoglobin respectively also contain such sequences in their 5′-flanking regions or within introns (Grandbastien *et al.*, 1986; Brown *et al.*, 1984; Lee and Verma, 1984). The significance of simple sequences for an enhancer effect in gene regulation is unknown. However, it can be speculated that their possible function is related to the special stereochemical and torsional properties of the DNA helix and its potential to form cruciforms (Suggs and Wagner, 1986; Alexeev *et al.*, 1987; McClellan *et al.*, 1986; Koo *et al.*, 1986; Wright and Dixon, 1986). A new and exciting possibility could be the nuclear scaffold attachment of large chromosomal loops via "scaffold attachment regions" (SARs) that also contain A- and T-rich motifs; such sequences cohabit with some transcriptional enhancers in *Drosophila* (Gasser and Laemmli, 1986; 1987). The enhancer function may be viewed as a structural DNA effect keeping the nearby genes in close contact with the transcription machinery in the nuclear matrix (Jackson, 1986). Studies of the DNA : protein interaction in DNA regions with functional significance will be of great importance in elucidating the molecular mechanism of enhancer function.

III. General Conclusions

Small hsps (15—20 kDa) are the characteristic and most abundant stress proteins in plants following hyperthermic treatment. Their synthesis correlates with the development of thermotolerance, which may also be induced by other stresses (e.g. arsenite and cadmium). A number of the small hsps share homologous sequences ($\sim 90\%$ for members of the same gene family, $< 50\%$ for different families) in soybean; their genes are also related to hs genes in other plants. Common (shared) characteristics in the predicted secondary structures are particularly pronounced in the C-terminal halves of these proteins. Although the function of the small hsps is unknown, their possibly protective role may be connected with their ability to aggregate or to associate with important cellular macromolecules.

The synthesis of hsps is primarily regulated by a transcriptional control allowing rapid induction of high levels of mRNA and a preferential translation during hyperthermia.

Local homologies in the promoter and 5′-flanking regions of soybean hs genes can be assigned to two different types of conserved sequences: (i) short palindromic sequences with 70% or more identity with the *Drosophila* consensus HSE sequence, and (ii) "simple sequences" (runs of A, T or AT) which are present in most genes upstream from the HSE-containing regions. Functional analysis following progressive deletions from one gene suggests a bipartite promoter structure. Whereas the proximal HSE-containing sequences are required for thermoregulated transcription, the more distally located region including some of the simple sequences is necessary for maximal enhancement of transcription in transgenic tobacco plants. It appears that the HSEs interact with a hs transcription factor,

promoting transcription in a way very similar to the HSTF : HSE interaction in animal cells. The enhancer-like function of the upstream region of a heat shock gene is without precedent. The binding of nuclear proteins to sequences in this region (F. Schöffl and K. Severin, unpublished results) is a first step towards an understanding of the mechanism of enhancer functions in heat shock and other environmentally controlled gene expression.

Acknowledgements

We thank Bob Kosier for his critical reading of the manuscript and Sieglinde Angermüller for her technical assistance in the preparation of graphs. The research was supported by grants from the Deutsche Forschungsgemeinschaft to F. S. We would also like to thank Drs. Jill Winter, Elisabeth Vierling, Joe Key, Günter Strittmatter and Nam-Hai Chua, for providing unpublished information and preprints.

References

Alexeev, D. G., Lipanov, A. A., Skuratovskii, I. Y., 1987: Poly(dA)/poly(dT) is a B-type double helix with a distinctively narrow minor groove. Nature (Lond.) **325,** 821—823.

Anathan, J., Goldberg, A. L., Voellmy, R., 1986: Abnormal proteins serve as eukaryotic stress signals and trigger the activity of heat shock genes. Science **232,** 522—524.

Ayme, A., Tissières, A., 1985: Locus 67B of *Drosophila melanogaster* contains seven, not four, closely related heat shock genes. EMBO J. **4,** 2949—2954.

Baumann, G., Raschke, E., Bevan, M., Schöffl, F., 1987: Functional analysis of sequences required for transcriptional activation of a soybean heat shock gene in transgenic tobacco plants. EMBO J., **6,** 1161—1166.

Berry, J., Björkman, O., 1980: Photosynthetic response and adaption to temperature in higher plants. Ann. Rev. Pl. Physiol. **31,** 491—543.

Bienz, M., 1985: Transient and developmental activation of heat shock genes. Trends Biochem. Sci. **10,** 157—161.

Blackman, R. K., Meselson, M., 1986: Interspecific nucleotide sequence comparisons used to identify regulatory and structural features of the *Drosophila* hsp82 gene. J. Mol. Biol. **188,** 499—516.

Brown, G. G., Lee, J. S., Brisson, N., Verma, D. P. S., 1984: The evolution of a plant globin gene family. J. Mol. Evol. **21,** 19—32.

Brugge, J., Erikson, E., Erikson, R. L., 1981: The specific interaction of the Rous sarcoma virus transforming protein, $pp60^{src}$, with two cellular proteins. Cell **25,** 363—372.

Burdon, R. H., 1986: Heat shock and the heat shock proteins. Biochem. J. **240,** 313—324.

Burke, J. J., Hatfield, J. L., Klein, R. R., Mullet, J. E., 1985: Accumulation of heat shock proteins in field grown cotton. Pl. Physiol. **78,** 394—398.

Catelli, M. G., Binart, N., Jung-Testas, I., Renoir, J. M., Baulieu, E. E., Feramisco,

J. R., Welch, W. J., 1985: The common 90 kd protein component of non-transformed "8S" steroid receptors is a heat shock protein. EMBO J. **4,** 3131—3135.

Cooper, P., Ho, T.-D., Hauptman, R. M., 1984: Tissue specificity of the heat shock response in maize. Pl. Physiol. **75,** 431—441.

Coldrion-Raichlen, P., Dietrich, P. S., Sinibaldi, R. M., 1986: The heat shock response of *Arabidopsis thaliana.* J. Cell Biol. **103,** 176a.

Czarnecka, E., Edelmann, L., Schöffl, F., Key, J. L., 1984: Comparative analysis of physical stress responses in soybean using cloned cDNAs. Pl. Molec. Biol. **3,** 45—58.

Czarnecka, E., Gurley, W. B., Nagao, R. T., Mosquera, L. A., Key, J. L., 1985: DNA sequence and transcript mapping of a soybean heat shock gene encoding a small heat shock protein. Proc. Natl. Acad. Sci. U. S. A. **82,** 3726—3730.

De Jong, W. W., 1982: Eye lens proteins and vertebrate phylogeny. In: Macromolecular sequences in systematic and evolutionary biology. pp. 75—114. Goodman, M. (ed.). Plenum Press, New York.

Dietrich, P. S., Bouchard, R. A., Sinibaldi, R. M., 1986: Isolation of maize 83, 70 and 18 kDa HS genes. J. Cell Biol. **103,** 311a.

Ellis, J. G., Llewellyn, D. J., Dennis, E. S., Peacock, W. J., 1987: Maize Adh-1 promoter sequences control anaerobic regulation: addition of upstream promoter elements from constitutive genes is necessary for expression in tobacco. EMBO J. **6,** 11—16.

Gasser, S. M., Laemmli, U. K., 1986: Cohabitation of scaffold binding regions with upstream enhancer elements of three developmentally regulated genes of *Drosophila melanogaster.* Cell **46,** 521—530.

Gasser, S. M., Laemmli, U. K., 1987: A glimpse at chromosomal order. Trends Genet. **3,** 16—22.

Glaczinski, H., Kloppstech, K., 1987: Heat induced alterations in the properties of thylakoid membranes. Europ. J. Cell Biol. **43,** 19.

Grandbastien, M. A., Berry-Lowe, S., Shirley, B. W., Meagher, R. B., 1986: Two soybean ribulose-1,5-bisphosphate carboxylase small subunit genes share extensive homology even in the distant flanking sequences. Pl. Molec. Biol. **7,** 451—465.

Guedon, G. F., Gilson, G. J. P., Ebel, J. P., Befort, N. M. T., Remy, P. M., 1986: Lack of correlation between extensive accumulation of bisnucleoside phosphates and the heat shock response in eukaryotic cells. J. Biol. Chem. **261,** 16459—16465.

Gurley, W. B., Hepburn, A. G., Key, J. L., 1979: Sequence organization of the soybean genome. Biochim. Biophys. Acta **561,** 167—183.

Gurley, W. B., Czarnecka, E., Nagao, R. T., Key, J. L., 1986: Upstream sequences required for efficient expression of a soybean heat shock gene. Molec. Cell. Biol. **6,** 559—565.

Hickey, E., Brandon, S. E., Potter, R., Stein, J., Weber, L. A., 1986: Sequence and organization of genes encoding the human hsp27 kDa heat shock protein. Nucl. Acids Res. **14,** 4127—4145.

Hoffman, E. P., Gerring, S. L., Corces, V. G., 1987: The ovarian, ecdysterone and heat shock responsive promoters of *Drosophila* hsp27 gene react very differently to perturbations of DNA sequence. Molec. Cell. Biol. **7,** 973—981.

Hultmark, D., Klemenz, R., Gehring, W. J., 1986: Translational and transcriptional control elements in the untranslated leader of the heat shock gene hsp22. Cell **44,** 429—438.

Ingolia, T. D., Craig, E. A., 1982: Four small *Drosophila* heat shock proteins are

related to each other and to mammalian α-crystallin. Proc. Natl. Acad. Sci. U.S.A. **79,** 2360—2364.

Jackson, D. A., 1986: Organization beyond the gene. Trends Biochem. Sci. **11,** 249—252.

Jones, D., Russnak, R. H., Kay, R. J., Candido, E. P. M., 1986: Structure, expression and evolution of the heat shock gene locus in *Caenorhabditis elegans* that is flanked by repetitive elements. J. Biol. Chem. **261,** 12006—12015.

Kalish, F., Cannon, C., Brunke, K., 1986: Characterization of a putative hsp81 gene in *Brassica oleracea* which is both constitutive and inducible. J. Cell. Biol. **103,** 176a.

Karin, M., Haslinger, A., Holtgreve, H., Richards, R. I., Krauter, P., Westphal, H. M., Beats, M., 1984: Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce human metallothionein IIa gene. Nature (Lond.) **308,** 513—519.

Kee, S. C., Nobel, P. S., 1986: Concomitant changes in high temperature tolerance and heat shock proteins in desert succulents. Pl. Physiol. **80,** 596—598.

Key, J. L., Czarnecka, E., Lin, C.-Y., Kimpel, J., Mothershed, C., Schöffl, F., 1983: A comparative analysis of the heat shock response in crop plants. In: Current topics in plant biochemistry and physiology. Vol. 2, pp. 107—118. Randall, D. D., Blevins, D. G., Larson, R. L., Rapp, B. J. (eds.). Columbia, Missouri. University of Missouri-Columbia.

Key, J. L., Gurley, W. B., Nagao, R. T., Czarnecka, E., Mansfield, M. A., 1985: Multigene families of soybean heat shock proteins. Vol. 83, pp. 81—100. Van Vloten-Doting, L., Groot, G., Hall, T. C. (eds.). Plenum Press, New York.

Kimpel, J. A., Key, J. L., 1985: Presence of heat shock mRNAs in field grown soybeans. Pl. Physiol. **79,** 672—678.

Kleinsek, D. A., Beattie, W. G., Tsai, M. J., O'Malley, B. W., 1986: Molecular cloning of a steroid-regulated 108 K heat shock protein gene from hen oviduct. Nucl. Acids Res. **14,** 10053—10069.

Klemenz, R., Hultmark, D., Gehring, W. J., 1985: Selective translation of heat shock mRNA in *Drosophila melanogaster* depends on sequence information in the leader. EMBO J. **4,** 2053—2060.

Kloppstech, K., Meyer, G., Schuster, G., Ohad, I., 1985: Synthesis, transport and localization of a nuclear coded 22 kDa heat shock protein in the chloroplast membranes of peas and *Chlamydomonas reinhardii.* EMBO J. **4,** 1901—1909.

Koo, H. S., Wu, H. M., Crothers, D. M., 1986: DNA bending at adenine/thymine tracts. Nature (Lond.) **320,** 501—506.

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. U.S.A. **83,** 8054—8058.

Lee, J. S., Verma, D. P. S., 1984: Structural and chromosomal arrangement of leghaemoglobin genes in kidney bean suggest divergence in soybean leghaemoglobin gene loci following tetraploidization. EMBO J. **3,** 2745—2752.

Lin, C.-Y., Roberts, J. R., Key, J. L., 1984: Acquisition of thermotolerance in soybean seedlings. Pl. Physiol. **74,** 152—160.

Lindquist, S., 1986: The heat shock response. Ann. Rev. Biochem. **55,** 1151—1191.

Lubben, T. H., Keegstra, K., 1986: Effective in vitro import of a cytosolic heat shock protein into pea chloroplasts. Proc. Natl. Acad. Sci. U.S.A. **83,** 5502—5506.

McClellan, J. A., Palecek, E., Lilley, M. J., 1986: $(AT)_n$ tracts embedded in random sequence DNA — formation of a structure which is chemically reactive and torsionally deformable. Nucl. Acids Res. **14,** 9291—9309.

McGarry, T. J., Lindquist, S., 1985: The preferential translation of *Drosophila* hsp70 mRNA requires sequences in the untranslated leader. Cell **42,** 903—911.

Mestril, R., Schiller, P., Amin, J., Klapper, H., Anathan J., Voellmy, R., 1986: Heat shock and ecdysterone activation of the *Drosophila melanogaster* hsp23 gene; a sequence element implied in developmental regulation. EMBO J. **5,** 1667—1673.

Munro, S., Pelham, H., 1985: What turns on heat shock genes? Nature (Lond.) **317,** 477—478.

Nagao, R. T., Czarnecka, E., Gurley, W. B., Schöffl, F., Key, J. L., 1985: Genes for low molecular weight heat shock proteins of soybean: sequence analysis of a multigene family. Molec. Cell. Biol. **5,** 3417—3428.

Nagao, R. T., Kimpel, J. A., Vierling, E., Key, J. L., 1986: The heat shock response: A comparative analysis. In: Oxford Surveys of Plant Molecular and Cell Biology. Vol. 3, pp. 384—438. Miflin, B. J. (ed.). Oxford University Press, Oxford, U. K.

Nover, L., Hellmund, D., Neumann, D., Scharf, K.-D., Serfling, E., 1984: The heat shock response of eukaryotic cells. Biol. Zbl. **103,** 357—435.

Nover, L., 1987: Expression of heat shock genes in homologous and heterologous systems. Enzyme Microb. Technol. **9,** 130—144.

Oppermann, H., Levinson, W., Bishop, J. M., 1981: A cellular protein that associates with the transforming protein of Rous sarcoma virus is also a heat shock protein. Proc. Natl. Acad. Sci. U.S.A. **78,** 1067—1071.

Ougham, H. J., Stoddart, J. L., 1986: Synthesis of heat shock protein and acquisition of thermotolerance in high-temperature tolerant and high-temperature susceptible lines of sorghum. Plant Sci. **44,** 163—167.

Pelham, H., 1985: Activation of heat shock genes in eukaryotes. Trends Genet. **1,** 31—35.

Pelham, H., 1986: Speculation on the function of the major heat shock and glucose regulated proteins. Cell **46,** 959—961,

Pelham, H. R. B., Bienz, M., 1982: A synthetic heat shock promoter element confers heat-inducibility on the herpes simplex virus thymidine kinase gene. EMBO J. **1,** 1473—1477.

Raschke, E., 1987: Molekulare Analyse verschiedener Gene für kleine Hitzeschockproteine der Sojabohne (*Glycine max* [L.] Merrill). Dissertation, Universität Bielefeld, F.R.G.

Raschke, E., Baumann, G., Schöffl, F., 1988: Nucleotide sequence analysis of soybean small heat shock protein genes belonging to two different multigene families. J. Mol. Biol. **199,** 549—557.

Roberts, J. K., Key, J. L., 1985: Characterization of the genes for the heat shock 70kD and 80kD proteins in soybean. First International Congress of Plant Molecular Biology, Savannah, GA, p. 137.

Rochester, D. E., Winter, J. A., Shah, D. M., 1986: The structure and expression of maize genes encoding the major heat shock protein, hsp70. EMBO J. **5,** 451—458.

Sachs, M., Ho, T. H., 1986: Alteration of gene expression during environmental stress in plants. Ann. Rev. Plant Physiol. **37,** 363—376.

Sargan, D. R., Tsai, M. J., O'Malley, B. W., 1986: Hsp108, a novel heat shock inducible protein of chicken. Biochem. **25,** 6252—6258.

Schlesinger, M. J., 1986: Heat shock proteins: the search for function. J. Cell Biol. **103,** 321—325.

Schlesinger, M. J., Ashburner, M., Tissières, A., 1982: Heat shock from bacteria to man. Cold Spring Harbor Laboratory, NY.

Schöffl, F., Baumann, G., 1985: Thermoinduced transcripts of a soybean heat shock gene after transfer into sunflower using a Ti plasmid vector. EMBO J. **4,** 1119—1124.

Schöffl, F., Raschke, E., Nagao, R. T., 1984: The DNA sequence analysis of soybean heat shock genes and identification of possible regulatory elements. EMBO J. **3,** 2491—2497.

Schöffl, F., Baumann, G., Raschke, E., Bevan, M., 1986: The expression of heat shock genes in higher plants. Phil. Trans. R. Soc. Lond. **B 314,** 453—468.

Schöffl, F., Rossol, I., Angermüller, S., 1987: Regulation of the transcription of heat shock genes in nuclei from soybean (*Glycine max*) seedlings. Plant Cell Envir. **10,** 113—119.

Singh, R. J., Hymowitz, T., 1985: The genomic relationship among six wild perennial species of the genus *Glycine* subgenus *Glycine willd.* Theor. Appl. Genet. **71,** 221—230.

Sinibaldi, R. M., Turpen, T., 1985: A heat shock protein is encoded within mitochondria of higher plants. J. Biol. Chem. **260,** 15382—15385.

Southgate, R., Ayme, A., Voellmy, R., 1983: Nucleotide sequence analysis of the *Drosophila* small heat shock gene cluster 67 B. J. Mol. Biol. **165,** 35—57.

Spena, A., Hain, R., Ziervogel, U., Saedler, H., Schell, J., 1985: Construction of a heat-inducible gene for plants. Demonstration of heat-inducible activity of the *Drosophila* hsp70 promoter in plants. EMBO J. **4,** 2739—2743.

Spena, A., Schell, J., 1987: The expression of a heat-inducible chimeric gene in transgenic tobacco plants. Mol. Gen. Genet. **206,** 436—440.

Strittmatter, G., Chua, N.-H., 1987: Artificial combination of two cis regulatory elements generates a unique pattern of expression in transgenic plants. Proc. Natl. Acad. Sci. U.S.A. **84,** 8986—8990.

Struhl, K., 1985: Naturally occurring poly(dA-dT) sequences are upstream promoter elements for constitutive transcription in yeast. Proc. Natl. Acad. Sci. U.S.A. **82,** 8419—8423.

Suggs, J. W., Wagner, R. W., 1986: Nuclease recognition of an alternating structure in $d(AT)_{14}$ plasmid insert. Nucl. Acids Res. **14,** 3703—3716.

Süss, K.-H., Yordanov, I. T., 1986: Biosynthetic cause of in vivo acquired thermotolerance of photosynthetic light reactions and metabolic responses of chloroplast to heat stress. Pl. Physiol. **81,** 192—199.

van den Heuvel, R., Hendriks, W., Quax, W., Bloemendahl, H., 1985: Complete structure of the hamster α A crystallin gene. J. Mol. Biol. **185,** 273—284.

Vierling, E., Mishkin, M. L., Schmidt, G. W., Key, J. L., 1986: Specific heat shock proteins are transported into chloroplasts. Proc. Natl. Acad. Sci. U.S.A. **83,** 361—365.

Vierling, E., Chen, Q., 1987a: Characterization and expression of chloroplast heat shock proteins in pea and petunia (in press).

Vierling, E., Roberts, J. K., Nagao, R. T., Key, J. L., 1987b: A chloroplast heat shock protein has homology to cytoplasmic heat shock proteins (in press).

Wright, J. M., Dixon, G. H., 1986: Induction by torsional stress of a cruciform conformation 5′ upstream of the gene for a high mobility group protein from trout and the specific binding to flanking sequences by the gene product. J. Cell Biol. **103,** 43 a.

Wu, B. J., Morimoto, R. I., 1985: Transcription of the human hsp70 gene is induced by serum stimulation. Proc. Natl. Acad. Sci. U.S.A. **82,** 6070—6074.

Xiao, C.-M., Mascarenhas, J. P., 1985: High temperature-induced thermotolerance in pollen tubes of *Tradescantia* and heat shock proteins. Pl. Physiol. **78,** 887—890.

Yost, A. J., Lindquist, S., 1986: RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. Cell **45,** 185—193.

Zhang, H., Croes, A. F., Liskens, H. F., 1984: Qualitative changes in protein synthesis in germinating pollen in *Lilium longiflorum* after a heat shock. Pl. Cell Envir. **7,** 689—691.