Plant heat shock transcription factors: divergence in structure and function

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1. Introduction

Heat shock transcription factor (HSF) is an important player in the signal transduction pathway that starts with the perception of molecular cues associated with high temperature stress, and leads to the elevated production of heat shock proteins (HSPs) and chaperones which protect the cell from the acute or prolonged effects of stress. The heat shock response is common to all living organisms and is a highly conserved one, as is the transcription factor that regulates it, HSF. Since the first discovery of the heat shock phenomenon in 1962 by Ritossa (1), a volume of information has been collected regarding yeast, *Drosophila*, mammalian, and plant HSFs. In the following review, we will primarily concentrate on plant HSFs and examine animal HSF1 as a paradigm for the mechanism of HSP gene regulation.

2. Activation of mammalian HSF

Induction of heat shock protein (HSP) genes is achieved by activation of preexisting HSFs, which then bind to the promoter and stimulate transcription. In animals, the activation of HSF from its repressed state in unstressed cells is a multistep process that can be separated into four phases: 1) oligomerization of inactive monomers to trimers, 2) nuclear localization, 3) high affinity DNA binding at the promoter, and 4) acquisition of transcriptional competence. In unstressed cells, mammalian HSF1 is located predominantly in the cytoplasm, but is also present in the nucleus. After exposure to protein damaging stresses, HSF1 undergoes a conversion to a trimeric state and acquires DNA binding activity (2). The regulation of trimerization involves two portions of the HSF1 protein, hydrophobic repeat C located near the C-terminus (3,4) and the other located within the oligomerization domain (4). Inactive HSF is thought to exist in a folded conformation through interactions between these two domains. One or more heat shock proteins have been implicated in maintaining the folded inactive state (5).

Although early models suggested that HSP70 directly controls HSF activity (6-8), recent evidence from Voellmy's group indicates that HSP90 may be the primary molecule regulating trimerization and activation of DNA binding of HSF1 in animals (9). The revised model for HSF1 activation now has HSP90 (or a multichaperone complex containing HSP90) bound in equilibrium to HSF1 under nonstress conditions to maintain the inactive state. During heat stress, free pools of HSP90 are depleted by interactions with denatured cellular proteins resulting in the unfolding of HSF1. The unfolded form is transported from the cytoplasm to the nucleus where it binds the heat shock consensus element (HSE) in the promoters of HSP genes as a trimer.

In mammals, the last step in the activation of HSF1 is the acquisition of transcriptional competence. Evidence for this final step is derived from the observation that certain anti-inflammatory drugs, such as salicylate and oxidative stress, induce HSF1 to trimerize and bind to HSEs; however, no transcription of heat shock genes occurs (10,11). A negative regulatory domain located between the oligomerization domain and a C-terminal transcriptional activation domain is thought to repress the function of HSF activation domains by masking (12-14). Little is known regarding the mechanism whereby transcriptional competence is achieved; however, a correlation between heat inducible phosphorylation of HSF1 and transcriptional activity has been shown (15,16).

3. Two classes of plant HSFs

The first cDNA for plant HSF was isolated almost a decade ago by Scharf and Nover (17), and since then a number of other HSF cDNAs and genes have been characterized from various plant species (18-23). Analysis of con-

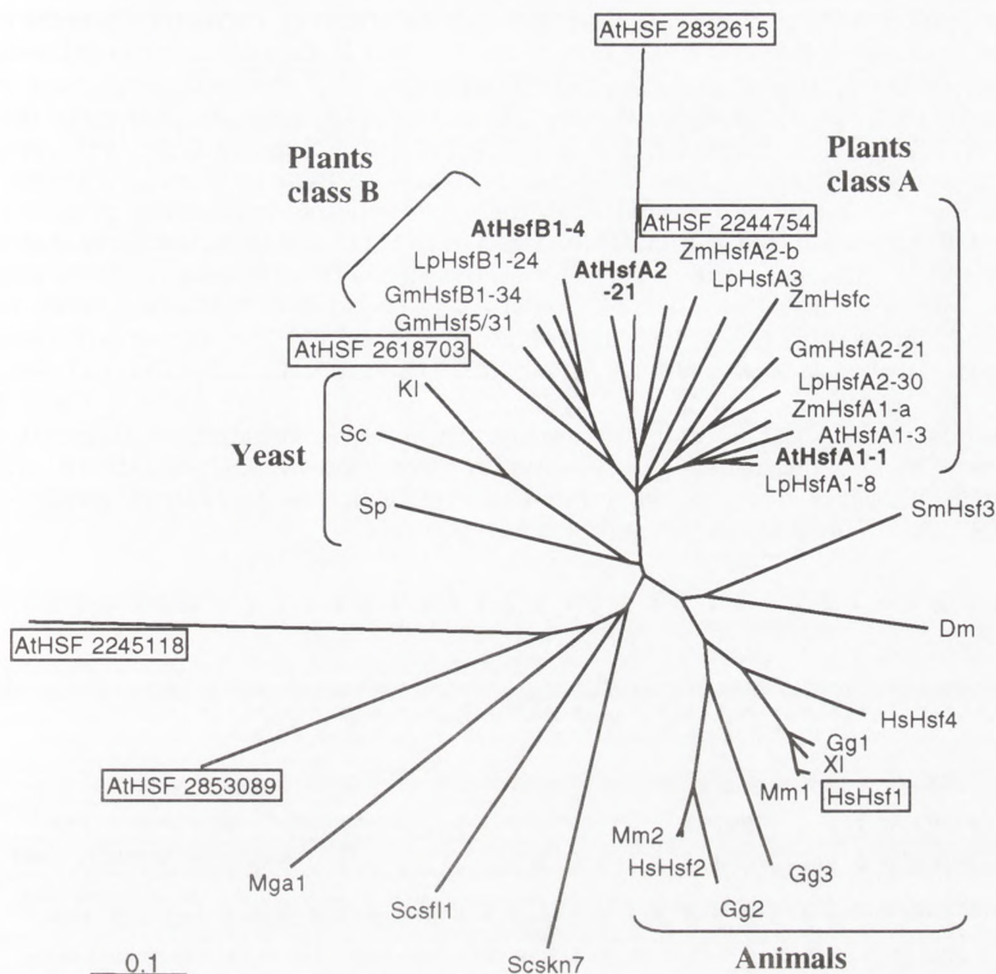

Fig. 1. HSF relatedness tree. The parsimony analysis of HSF DNA binding domains was conducted using PAUP software with 100 repetitions according to the bootstrap method at the 50% confidence level. Human HSF1 (boxed in) was used as a point of reference. Legend: Gm, *Glycine max*; At, *Arabidopsis thaliana*; Zm, *Zea mays*, Lp, *Lycopersicon peruvianum*. All sequences were acquired from publicly accessible data bases. The seven-digit numbers of five AtHSFs are the respective accession numbers of newly classified *Arabidopsis* HSFs (boxed in). The numbers or letters after the hyphen in HSF names are the original cDNA clone or gene designations.

served oligomerization domains and parsimony analysis of amino acid sequences of DNA binding domains indicates that the plant HSF family is distinct from that of yeast and animal HSFs (Fig. 1). Two well defined classes, A and B, can be discerned among plant HSFs (20,24). The lineage relationships among members of class A seem to be rather complex, while the class B HSFs seem to be derived from a common ancestor. Both classes contain

multiple members from various plant species. The key regulatory protein of the heat-inducible response in vertebrates, HSF1, cannot be grouped with any of the tested plant HSFs. Simple analysis of amino acid sequences of HSF DBDs does not identify plant HSFs that may play an analogous role during the stress response. It is possible that in plants, multiple HSFs may be specialized for the heat inducible response to high temperature stress.

Recent information from the *Arabidopsis* genome sequencing project is incorporated into the parsimony analysis of HSF DNA binding domains shown in Fig. 1 (22). It is quite evident that the *Arabidopsis* family of HSFs may consist of as many as nine, and most likely even more, HSFs (see Table 1). After eliminating nonrelated sequences, we classified the newest additions to the *Arabidopsis* HSF family, AtHSF2832615 and AtHSF2244754, to belong to the A2 subclass of class A HSFs, and AtHSF2618703 to be a class B representative that is distinct from the previously characterized AtHSFB1-4 (see Fig. 1 and Table 1). In addition, two clones, AtHSF2245118 and AtHSF2853089, seem to be related to the Scsfl1/Scskn7/Mga1 family of HSF-like factors identified, so far, only in yeast.

TABLE 1
CHARACTERIZED AND PUTATIVE HSFs FROM *Arabidopsis thaliana*

Name	Acc.#	Class	Reference
AtHSFA1-1	729773	A	Plant Mol. Biol., 26, 353-362, 1994
AtHSFA1-3	3256068	A	Mol. Gen. Genet., 258, 269-278, 1998
AtHSFA2-21	3399765	A (cDNA)	Barros et al., 1996, direct submission Cell Stress Chap. 1, 215-223, 1996
HSF-like[1] protein	2832616	AtHSFA2-21 (gene)	Bevan et al., 1998, direct submission
Homolog LpHSFA2-30	2244754	A	Bevan et al., 1997, direct submission Nature, 391, 485-488, 1998
HSF-like protein	2832615	A	Bevan et al., 1998, direct submission
AtHSFB1-4	2129612 1619921	B	Barros et al., 1996, direct submission, Cell Stress Chap. 1, 215-223, 1996
Putative HSTF	2618703	B	Rounsley et al., 1997, direct submission
Hypothetical protein	2245118	HSF-like	Bevan et al., 1997, direct submission, Nature, 391, 485-488, 1998
Putative protein	2853089	HSF-like	Bevan et al., 1998, direct submission

[1] genomic clone identical to AtHSFA2-21 3399765 cDNA
The protein sequence analyses were done using the BLASTP program. Numbers after the hyphen in HSF names in the left column are the original cDNA clone or gene designations.

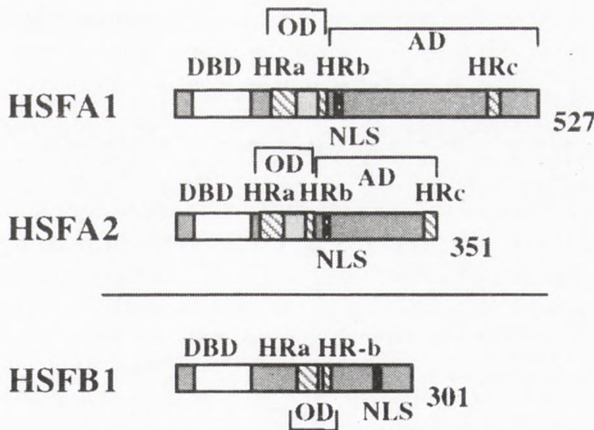

Fig. 2. Diagram of structural features of class A and B plant HSFs. Tomato HSFs, LpHSFA1-8, LpHSFA2-30 and LpHSFB1-24, were used as the representatives for individual HSF subfamilies, respectively (Scharf et al., 1990; Treuter et al., 1993). Legend: DBD, DNA binding domain; HR-a, -b, -c, hydrophobic heptapeptide repeats; AD, transcriptional activation domain; NLS, nuclear localization sequence.

4. Transcriptional activation potential of class A and B HSFs

The trend in class B HSFs has been a streamlining of C-terminal region, a loss of hydrophobic repeat c (HR-c) and the translocation of nuclear localization sequence (NLS) towards the C-terminus (Fig. 2). These changes in structure are indications of a possible functional differentiation between the two classes of plant HSFs. The first indications of such differences were obtained from HSF substitution studies done in yeast. Tomato LpHSFs, A1-8 and A2-30, as well as *Drosophila* HSF, can functionally replace endogenous yeast ScHSF, while class B HSF, LpHSFB1-24, is not able to substitute unless a short transcriptional activation motif from class A tomato HSF is fused to the C-terminus (25). Two soybean class B HSFs, GmHSFB1-34 and GmHSF5, had no transcriptional activity in yeast when monitored by an HSE/β-Gal reporter, but surprisingly, GmHSF5 was able to functionally substitute for ScHSF under control conditions, while soybean GmHSFB1-34 conveyed viability to yeast only if fused to a heterologous activation domain (26).

The lack of transcriptional activity of some HSFs was confirmed by studies in transgenic *Arabidopsis* plants. Overexpressed class A AtHSFA1-1 displayed heat-inducible regulation of DNA binding activity; however, when overexpressed in *Drosophila* or human cells, it showed constitutive DNA binding and transcriptional activities (27). Similar overexpression of another class A member, *Arabidopsis* AtHSFA1-3, in transgenic plants resulted in constitutive transcriptional activity and HSP synthesis at non-heat shock temperatures (23). Although two overexpressed AtHSFs were unregulated, one in animal cells (AtHSFA1-1) and the other in transgenic *Arabidopsis*

plants (AtHSFA1-3), the endogenous animal HSFs were still under tight heat-inducible regulation. These observations suggest that plant HSFs, unlike their mammalian counterparts, may acquire transcriptional competence automatically upon trimerization.

In contrast to results obtained with class A HSFs, plants overexpressing class B AtHSFB1-4, or the chimeric AtHSFB1-4/GUS construct, displayed no constitutive synthesis of HSPs (23). The analogous AtHSFA1-1/GUS chimeric construct was de-repressed in its heat-inducible regulation and directed constitutive production of HSPs (28). In cases of transgenic plants overexpressing genetically engineered class A HSF-GUS constructs, or AtHSFA1-3 without GUS, the constitutive synthesis of heat shock proteins can be linked to increased basal, but not acquired, thermotolerance.

Opposite results were obtained in transgenic plants overexpressing the HSP70 antisense gene (29). Only acquired thermotolerance was affected, and in a negative fashion: the threshold temperature of plant survival was lowered by 2°C as compared to the wild type or transgenics overexpressing AtHSFA1-1. Since in antisense HSP70 plants, AtHSFA1-1 still was under negative regulation, maintenance of the repressed state of HSF clearly does not depend on high levels of HSP70/HSC70 (the dependence previously implied for the heat regulation of animal HSF (6-8)). Instead, the time required to turn HSF activity off during recovery from heat stress was significantly prolonged in HSP70 antisense plants. This indicates the possibility that HSP70 may be involved in the disassembly of HSF trimers and may play a role in attenuation of the heat shock response (29). However, to date there is no evidence in plants of direct protein:protein interaction between HSP70 and HSF.

The cited studies document distinct functional differences between class A and B HSFs. Members of the A group are involved in the activation of the HS genes in response to environmental stresses. Work in our laboratory indicates that class B HSFs from *Arabidopsis* and soybean possess functional DNA binding domains, but either have no capacity to activate transcription, or very little transcriptional activity (26).

5. Organization of HSF functional domains

Mammalian HSFs contain six types of functional domains: 1) the DNA binding domain (DBD), 2) the oligomerization domain (OD), 3) a region that suppresses oligomerization (hydrophobic repeat c; HR-c), 4) transcriptional activation domains (ADs), 5) a nuclear localization sequence (NLS), and 6) a region involved in suppressing transcriptional activity (negative region; NR) (5). Of these, the most conserved are the DNA binding and the oligomerization domains.

5.1. DNA binding domain

The DBD is comprised of 118 aa residues and is located at the N-terminus. Even though it was not obvious from simple inspection of amino

acid sequence, structural studies revealed that it is related to the HNF3/*fork-head* class of the helix-turn-helix family of DNA binding motifs (30-34). The recognition helix makes contact with the major grove of the DNA (35-38), and each subunit of the trimer binds to one HSE core sequence (37). A feature distinctive of plant HSFs is the absence of 11 residues between β-strands 3 and 4 resulting in a turn of only four amino acids (24).

5.2. Oligomerization domain

The oligomerization domain consists of two groups of hydrophobic hepta-peptide repeats, the long and short arrays. The long array (HR-a) contains from 5 to 6 heptad repeats and is directly involved in formation of the triple-stranded α-helical coiled-coil (39). The short array (HR-b) contains two overlapping arrays of 3 to 4 heptad repeats and is thought to provide stabilization by buttressing the outside of the long array. A third array of hydrophobic heptad repeats (HR-c) is usually located near the C-terminus of the protein. Interestingly, the HR-c array is absent in human HSF4 (40) and is either absent or poorly conserved in the plant group B HSFs (Fig. 2). It is worth noting that hHSF4 is similar to plant class B HSFs since it has very little capacity to activate transcription and it inhibits the heat shock response when overexpressed (40).

In animal HSFs, the transition from monomer to trimer is an important step in the regulation of HSF DNA binding activity. The apparent affinity of the monomeric HSF for DNA is increased by approximately 10,000-fold by trimerization (41-43). Experiments with *Drosophila*, chicken and human HSFs have shown a release of negative regulation affecting trimerization obtained by deletion or alteration of the C-terminal HR-c region (3,4,44).

In plants, it has been shown that heat induces an increase in DNA binding to HSE probes using extracts from tomato, *Arabidopsis*, and soybean (17,19,45). In *Arabidopsis*, trimerization of endogenous HSF was shown to be correlated with heat-activated DNA-binding (29). From these results it appears that HSF trimerization is heat-inducible in plants for some of the class A HSFs, as in most other eukaryotes with the exception of budding yeast. However, this generalization must be viewed with some caution since, in tomato, the role of trimerization in nonstressed plants is not as clear. Lutz Nover and Dieter Scharf's group has been unable to see a difference in the trimerization state before and after heat shock (46). It is not known if trimerization is heat inducible for class B HSFs.

5.3. Transcriptional activation domain

The transcriptional activation domains of HSFs from yeast, *Drosophila*, mammals, and tomato have been mapped in detail. Two adjacent activation domains (AD1 and AD2) have been identified in the C-terminal portion of human HSF1 (13,47,48). AD1 encompasses 20 amino acids (aa 401 to 420) and is rich in bulky hydrophobic and acidic amino acids. AD2 (aa 431 to

529) is highly acidic and probably lacks helical secondary structure due to the large number of proline and glycine residues. *Drosophila* HSF is organized in a similar manner with a transactivation domain rich in hydrophobic and acidic residues located at the extreme C-terminus (49).

In tomato, three HSFs have been cloned, and in each case a tryptophan motif (trp-repeat) located C-terminally to the OD has been associated with transcriptional activity (50). This activation motif has been renamed the AHA (**a**romatic, bulky **h**ydrophobic, and **a**cidic residues) module due to its similarity to analogous regions located in animal and yeast HSFs that do not contain a tryptophan as the aromatic residue (46). This family of activation modules is widespread with similar sequences found in activators that are usually negative in charge like VP16, Jun, Gal4, RelA and others (46). Mutational studies with Gal4 (51) and with a tomato HSF (Nover and Doering, personal communication) suggest that the hydrophobic residues are more important than the negative charges.

5.4. Negative regulatory regions of mammalian HSF1

In addition to positive acting domains, mouse and human HSF1 contain a region that exerts negative control, preventing transcriptional activation under non-heat shock conditions. This negative regulatory domain is located predominantly between the OD and the C-terminal activation domains (13,14,47), but also includes the C-terminal portion of the OD (12). The NR in HeLa cells confers heat inducibility on both AD1 and AD2, but when expressed in yeast, it does not appear to be heat regulated (13). No similar domain has been identified in plant HSFs.

5.5. Functional analysis of the C-terminus of plant HSFs

A series of observations regarding deletions of the extreme C-terminus of plant HSFs suggests that these sequences are involved in the regulation of transcriptional activity; however, the details of the mechanism are still unclear. Using transient expression in tobacco mesophyll protoplasts and an HSE/ GUS reporter, we compared the inert class B HSFs from soybean and *Arabidopsis* with the three tomato HSFs, which are all active (26,50). All class B soybean HSFs and *Arabidopsis* AtHSFB1-4 were inert, while the two tomato class A HSFs and a class A2 *Arabidopsis*, AtHSFA2-21, showed normal activity (Fig. 3). All HSFs were tested for DNA binding activity using a reporter that contains HSEs downstream of the CaMV 35S TATAA motif (50). Overexpression of all HSFs resulted in clear inhibition of reporter activity, indicating that both class A and B HSFs were expressed and contained functional DNA binding domains (26).

The mapping of functional domains identified a short region in *Arabidopsis* AtHSFA2-21 that functions as an activation module and is similar to the AHA motif present in other plant class A HSFs. Deletion analysis of the class A HSFs, LpHSFA1-8 (tomato) and AtHSFA2-21 (*Arabidopsis*), failed to

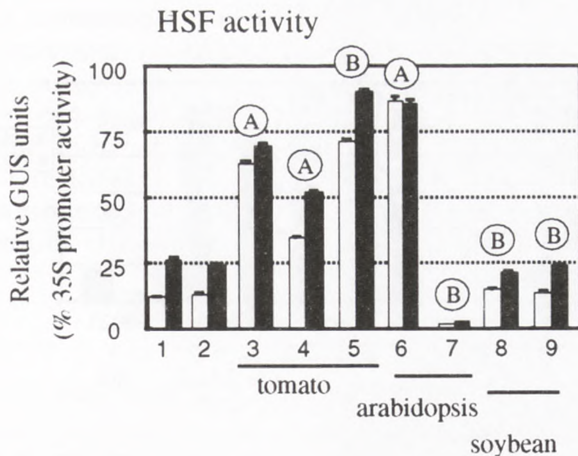

Fig. 3. Transcriptional activity of plant class A and B HSFs. Transient assays were conducted using tobacco protoplasts to monitor HSE/GUS promoter activity in response to overexpressed plant HSFs. Y-axis is % GUS reporter activity relative to the 35S CaMV promoter. Circled "A" and "B" indicate HSF classes. Lanes: 1 = endogenous HSFs; 2 = Gal4DBD (negative control); 3 = LpHSFA1-8; 4 = LpHSFA2-30; 5 = LpHSFB1-24; 6 = AtHSFA2-21; 7 = AtHSFB1-4; 8 = GmHSFB1-34; 9 = GmHSF5. Legend: room temperature = white bars; HS = black bars.

uncover regions that inhibited their own activity in a heat-inducible manner, suggesting that plant HSFs do not contain domains that exert negative control on transcription as is the case for animal HSFs (13,14,49). Interestingly, the GmHSFB1-34 C-terminal region strongly inhibited in *cis* expression of the human HSF1 activation domain fused to the C-terminus, but this inhibition was not heat regulated.

A controversial exception to the general finding that class B HSFs are inert is seen with tomato class B1 (Fig. 3; lane 5). Surprisingly, substitution studies in yeast indicated that LpHSFB1-24 was unable to support growth (25). In addition, the C-terminal region of tomato LpHSFB1-24 fused to Gal4DBD did not convey transcriptional activity to the GUS reporter driven by the minimal promoter and upstream Gal4 DNA binding sites (unpublished). The variable nature of tomato LpHSFB1-24 activity and the observation that not all class B HSFs in soybean show the same pattern of mRNA expression (discussed below) suggests that not all class B HSFs are completely devoid of transcriptional activity. For example, the apparent heat-induced degradation of GmHSF5 and GmHSF31 mRNAs may indicate that these factors act in a more specialized way than GmHSFB1-34, which appears to be the predominant class B HSF in soybean (20). Perhaps some of these less abundant HSFs maintain a low level of developmentally-specific expression for certain HSPs.

As a test of the ability of the group B HSFs to repress transcription at heat shock promoters, several class B HSFs were co-transformed with class

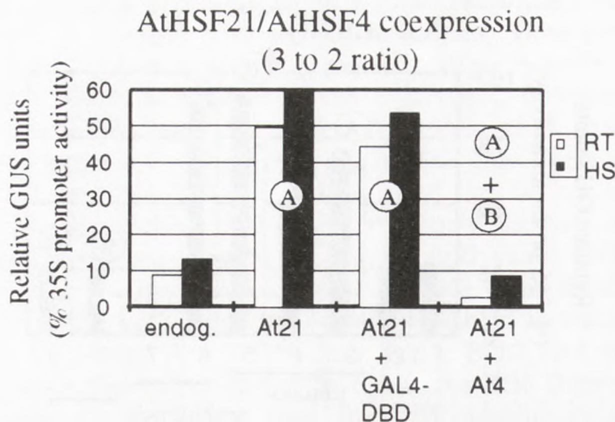

Fig. 4. Coexpression of *Arabidopsis* class A AtHSFA2-21 with class B AtHSFB1-4 resulted in *trans*-repression of AtHSFA2-21 activity. Transient assays were monitored using a HSE/GUS reporter in tobacco protoplasts. No significant effect was seen when the Gal4DBD was coexpressed with AtHSFA2-21. Y-axis is % GUS reporter activity relative to 35S CaMV promoter. Legend: room temperature = white bars; HS = black bars.

A HSFs. Coexpression at a 3:2 ratio of class A to class B effector DNAs resulted in strong *trans*-inhibition of activity for class A HSFs using an HSE-driven promoter/GUS reporter (Fig. 4; example using *Arabidopsis* HSFs and (26)). The implications of both types of repression, *intra*-molecular or "*cis*-repression", and *inter*-molecular or *trans*-repression, exhibited by some group B HSFs are still unclear but may indicate that some of the class B HSFs have the capacity to inhibit transcription through an active mechanism instead of the passive mode of simple promoter occupancy.

6. The complexities of transcriptional regulation of the heat shock response

In plants, the different classes of HSP genes are often present as multifamily groups that vary in their patterns of expression at the mRNA level. This complexity in expression is poorly understood, but may reflect differences in the organization of HSEs in the promoter and the possible involvement of HSF-independent pathways of activation, especially for some of the HSP70 genes. Another layer of complexity is added in plants if one considers the wide array of HSF genes present in plants and the differing patterns of heat inducibility of plant HSF mRNAs (Table 2). The existence of multiple HSFs suggests that plant HSFs have adapted specialized roles. In class A HSFs, two general patterns of mRNA expression are evident across various plant species: one group is comprised of constitutively expressed HSFs where ex-

pression is not influenced by heat shock, and the other HSFs show no detectable mRNA in control tissues, but their mRNAs are strongly heat-inducible. These expression patterns seem to support division of class A HSFs into two putative subgroups, A1 and A2. In class B HSFs, two patterns of mRNA expression can be elucidated as well: those that are present in unstressed tissue and induced by elevated temperature to very high levels (e.g. AtHSFB1-4 and GmHSFB1-34), and those that are constitutively expressed but their mRNAs undergo degradation during the heat or other stress treatments, e.g. cadmium chloride (GmHSF5 and 31) (20).

TABLE 2

mRNA EXPRESSION PATTERNS OF CLASS A AND B HSFs

Name	Plant material	Expression		Reference
		C	HS	
LpHSFA1-8	Tomato cell culture	+	+	Scharf et al., 1990
AtHSFA1-1	Whole Arabidopsis plants	+	**2+**	Hubel et al., 1994
AtHSFA1-3	Arabidopsis leaves	+	+	Praendl et al., 1998
ZmHSFA1-a	Maize leaves from 10 days old seedlings	+	+	Gagliardi et al., 1995
ZmHSFA1-b	Maize leaves from 10 days old seedlings	–	**2+**	Gagliardi et al., 1995
ZmHSFc	Maize leaves from 10 days old seedlings	–	+	Gagliardi et al., 1995
LpHSFA2-30	Tomato cell culture	–	**3+**	Scharf et al., 1990
GmHSFA2-21	Ethiolated soybean seedlings (2-3 cm)	–	**3+**	Czarnecka-Verner et al., 1995
AtHSFB1-4	Arabidopsis leaves	+	**5-10+**	Barros et al., 1996, GeneBank submission Praendl et al., 1998
LpHSFB1-24	Tomato cell culture	+	**2-3+**	Scharf et al., 1990
GmHSFB1-34	Ethiolated soybean seedlings (2-3 cm)	+	**5-10+**	Czarnecka-Verner et al., 1995
GmHSF29	Ethiolated soybean seedlings (2-3 cm)	+	**5+**	Czarnecka-Verner et al., 1995
GmHSF33	Ethiolated soybean seedlings (2-3 cm)	+	**3+**	Czarnecka-Verner et al., 1995
GmHSF5	Ethiolated soybean seedlings (2-3 cm)	+	+/–	Czarnecka-Verner et al., 1995
GmHSF31	Ethiolated soybean seedlings (2-3 cm)	+	+/–	Czarnecka-Verner et al., 1995

Numbers after the hyphen in HSF names in the left column are the original cDNA clone or gene designations.

A study of stress-induced expression of tomato HSFs presented by Scharf and colleagues (52) provides interesting insights regarding HSF expression.

Although mRNA levels of constitutive LpHSFA1-8 declined at the beginning of a HS treatment, these could quickly recover during medium to high temperatures (33 – 35°C); however, exposure to severe stress (39 – 40°C) caused a marked delay in the recovery of pre-exposure mRNA levels. This delay was shortened by pretreating the cells with high temperatures. In addition, severe stress seemed to reduce the heat inducibility of LpHSFB1-24 unless proceeded by prestress. In contrast, LpHSFA2-30 responded to the extreme heat stress by a proportionate increase in the mRNA synthesis without a delay period and, thus, may be adapted for high temperature stresses.

The simple heat stress induction of HSF expression can potentially be further complicated by temporal regulation during plant development or may be tissue specific. For example, in the absence of heat shock, maize ZmHSFA1-a was detected throughout the five stages of microgametophyte development, even in cells that were unable to trigger the heat shock response, such as mid-tricellular stage cells or mature pollen (21). A similar pattern was shown for ZmHSFA2-b, but no expression was seen in mature pollen. In contrast to expression in leaves, neither of the heat inducible maize HSFs (b and c) was significantly enhanced by heat stress at any stage of pollen development. Overall, heat-induced accumulation of HSP transcripts was much weaker during pollen development than in vegetative tissues which is consistent with the lack of the heat inducibility of HSF genes in immature pollen.

7. Heat shock granules and the functional interdependency of two tomato class A HSFs

A general feature of plant cells is the appearance during HS of electron dense material forming 40 nm-diameter ribonucleoprotein (RNP) aggregates in the cytoplasm. These heat shock granules (HSG) are the major sites of HSP accumulation (53). In tomato cell cultures, the HSG fraction contains HSP17 and, surprisingly, heat shock transcription factor LpHSFA2-30 (54). No other cellular proteins such as tubulin, HSP90, LpHSFA1-8 or LpHSFB1-24 are present.

The intracellular localization of various HSFs differs for the individual HSFs and in some cases shows a rather complex pattern. For example, in tomato cell cultures under non-HS conditions, the constitutively expressed LpHSFA1-8 is distributed between the nucleus and the cytoplasm (54). With elevated temperature it migrates to the nucleus and subsequently returns to the cytoplasm during the recovery from HS. Tomato class B HSF, LpHSFB1--24, is always found in the nucleus, irrespective of the temperature; however, a larger accumulation in the nucleus is evident after heat shock. Conversely, another representative of class A, LpHSFA2-30, is not found in control cells but is rapidly synthesized with the onset of the stress response and localizes predominantly in the cytoplasm. After the second heat shock, nearly all of it is transported to the nucleus. The sequestration of LpHSFA2-30 in gran-

ules is a dynamic, recovery-reversible process. In transformed and heat stressed tobacco protoplasts, overexpressed LpHSFA2-30 (despite the presence of an NLS) is defective in nuclear transport and accumulates in cytoplasmic HSGs. However, it localizes to the nucleus when coexpressed with LpHSFA1-8, but not LpHSFB1-24. The import of LpHSFA2-30 to the nucleus depends on direct physical interaction with LpHSFA1-8 which occurs via the oligomerization domains of both HSFs. The minimal region required for this heterologous association between both HSFs consists of hydrophobic repeat b (HR-b) of the OD and a functional NLS, while the transcription activation domain of LpHSFA1-8 seems to be dispensable for this process.

Lyck et al., (55) deleted either 8 or 28 aa residues from the C-terminus (within HR-c) of tomato LpHSFA2-30 and found that the overexpressed protein was more active and was transported to the nucleus regardless of the temperature; whereas before deletion, it was found to be mostly associated with granules in the cytoplasm. This observation implicates these deleted C-terminal residues in regulating associations between HSFA1 and HSFA2. Studies done in our laboratory are consistent with this finding in that transcriptional activity of *Arabidopsis* AtHSFA2-21 is increased (ca. 50%) upon deletion of the extreme C-terminus. However, the functional interdependency of AtHSFA2-21 with another HSF for nuclear import has yet to be determined (26).

The heat shock-inducible HSF1 granules in animal cells differ from those found in plants in several aspects. First, they are detected predominantly in the nucleus of heat stressed HeLa cells, not in the cytoplasm (56). Second, they are much smaller than plant granules ranging from 0.5 to 2.5 μm. They also appear to form two distinct sub-populations, and can be distinguished from nuclear speckles which contain other HSFs (HSF2, 3 and 4) and are not heat inducible. HSF1 granules display three distinct types of morphology, and although they co-localize with newly synthesized pre-mRNA induced by heat shock (57), they do not co-localize with specific HSP transcripts for HSP70, HSP90alpha and HSP90beta (58). The appearance of HSF1 granules correlates with the acquisition of HSF1 DNA binding activity, the appearance of the inducibly phosphorylated form of HSF1, and with the acquisition of transcriptional competence (56). Specific MAP kinases seem to down regulate the transcriptional activity of hHSF1 after heat shock and facilitate the disappearance of HSF1 granules (57). Interestingly, there seems to be a clear relationship between the number of HSF1 foci and the ploidy of the cell that implies that distinct chromosomal targets exist for HSF1 during heat shock.

Various groups have suggested that HS granules may function in recycling of HSF. It seems that in plants, HSGs serve primarily to accumulate partially denatured proteins and mRNAs, while in animals granule association with HSF1 seems to play a larger role.

Alternatively, HSF granules may have no specific function, but rather, represent aggregations of HSF1 that are formed by the transcriptionally active state of the protein. Perhaps exposed C-terminal activation domains

bind each other due to their hydrophobic residues as activated HSF accumulates to high levels after heat shock. Under this scenario, the HSF1 granules are a byproduct of activation but not directly involved in regulation of the HS response.

8. The mechanism of regulation of heat shock response in plants

In plants, HSFs belong to two major lineage groups: activators of class A, and class B HSFs which seem mostly inert. Within the class A activators, variations in function are evident. Some factors may serve as major stress-responsive HSFs, while others may be auxiliary and simply boost the activity of the primary HSFs as exemplified by the co-dependency of two tomato HSFs, LpHSFA1-8 and LpHSFA2-30 (54).

It seems that the majority of plant class B HSFs, while capable of binding to HSEs in the HS promoter, have very little or no capacity to activate transcription (20,26). The general occurrence and evolutionary preservation of class B HSFs in plants strongly argues that they play an important biological role in plants. This expectation is supported by the finding of a similar class in humans that has residual transcriptional activity (HSF4 (40)). Based on these assumptions, we propose that many of the class B HSFs play a role in negatively regulating the HS response, either in maintaining basal repression, or in attenuating the response after the initial burst of transcriptional activity (26,59).

According to the proposed model for HSF function, HSP gene promoters are maintained in the inactive state under nonstress conditions by class B HSFs in order to prevent fortuitous HSP gene expression. This is thought to occur through the binding of the constitutively trimerized and inert class B HSFs to the promoter. The lack of a C-terminal hydrophobic repeat in class B HSFs correlates with the prediction that class B HSFs are constitutively trimerized. Also, the finding that tomato LpHSFB1-24 is present in the nucleus in control cells is consistent with this view. If the animal paradigm for HSF1 holds for plants, class A activator HSFs should reside in the cytoplasm in an inactive state under basal conditions, perhaps as monomers held in a folded conformation by *intra*-molecular interactions and by *inter*-molecular interactions with molecular chaperones *i.e.* HSP90. However, Nover's group has been unable to demonstrate the existence of monomeric HSF forms in tomato cells or in tobacco protoplasts during transient expression assays, nor HS-dependent changes in oligomeric state of plant HSFs (54). The activation of HSP gene promoters depends on the binding of the transcriptionally competent class A HSFs to HSEs upstream of the TATA motif. In plants, many HS promoters may be occupied by class B HSFs under nonstress conditions. Although this prediction awaits experimental confirmation, the presence of tomato LpHSFB1-24 in the nucleus in nonstressed cells is consistent with class B HSFs occupying HS promoters prior to heat induction (54).

The mechanism behind this postulated replacement of class B HSFs with class A HSFs is a matter for further speculation. One possibility is that the two classes of HSF show differences in cooperativity in binding to clusters of HSEs. Our prediction is that B-HSFs exhibit much less cooperativity in binding than A-HSFs. Under this scenario, the class B HSFs would occupy the promoter under nonstress conditions by binding to single well-conserved core HSEs. Upon HS, the level of activated A-HSFs would rapidly increase in the nucleus. The A-HSFs would out-compete the B-HSFs through the advantage conferred by cooperativity in binding to the HSE clusters. The highly cooperative class A HSFs would bind to both perfect and imperfect HSE cores and would quickly occupy all HSEs on the promoter. A precedence for differential cooperativity existing between HSFs is seen in mammals in the differences between HSF1, which is highly cooperative, and HSF2, which prefers to bind as a single trimer (60). Little is known in plants regarding the kinetics of HSF binding; however, a report by Shimizu et al., (61) may have relevance. For example, two types of HSE binding activities were reported in tobacco: one that is constitutive and non-cooperative (class B?), and another that is heat inducible and cooperative (class A?).

The final step in HSF activation in mammals involves the derepression of the C-terminal transcriptional activation domains. The lack of a negative regulatory region in class A HSFs suggests that this mode of regulation is absent in plants. Instead, this check on fortuitous activation of HS genes may involve the interplay between class A and B HSFs for occupancy of HS promoter. We postulate that the inert class B HSFs serve to prevent accidental activation of HS genes by binding to HS promoters under nonstress conditions. This inhibition of premature activation of HS promoters may also sharpen the transition from the inactive to activated state of the promoter due to the cooperative nature of class A interactions at the promoter. The process whereby class B HSFs regain promoter occupancy during the recovery stage of the stress response is even more open to speculation; however, HSF70 seems to play a role. The removal of HSP70 in antisense transgenic plants clearly indicated such involvement, since in plants without HSP70 dissociation of the HSF trimer it occurred approximately 4-fold slower (29).

Many of the mRNAs for class B HSFs are present under nonstress conditions and are inducible to higher levels upon HS, a pattern of expression that sets them apart from animal and fungal HSFs (Table 2) (20,46). The heat inducibility of class B HSFs is also consistent with their postulated role in the attenuation of the HS response. Produced in large quantities during HS, inert HSFs are available to replace activator HSF at the promoter causing the attenuation of heat shock response. Although the mode of regulation proposed for class B HSFs may not be unique to plants, based on possible parallels with human HSF4, it seems highly elaborated in plants, perhaps due to their sedatory life style making it difficult to escape extremes in temperature.

At present the involvement of inert HSFs in regulation of the heat shock response is still only hypothetical and the predictions of this hypothesis

need experimental verification. The interplay of repressor and activator HSFs we anticipate to occur in plants may have useful applications in the engineering of promoter expression and in genetic strategies to extend the environmental range of crop species.

Abbreviations

Abbreviations used in current text have been shown alphabetically:
AD — transcriptional activation domain
CaMV — cauliflower mosaic virus
DBD — DNA binding domain
GUS — β-glucuronidase
HR — hydrophobic heptapeptide repeats a-, b- and c
HS — heat shock
HSC — heat shock cognate
HSF — heat shock transcription factor
HSP — heat shock protein
NLS — nuclear localization signal
NR — negative regulatory region
OD — oligomerization domain

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Literature

1. Ritossa F., (1964), Exp. Cell Res., 35, 601-607.
2. Baler R., Dahl G., Voellmy R., (1993), Mol. Cell. Biol., 13, 2486-2496.
3. Rabindran S. K., Haroun R. I., Clos J., Wisniewski J., Wu C., (1993), Science, 259, 230-234.
4. Zuo J., Baler R., Dahl G., Voellmy R., (1994), Mol. Cell Biol., 14, 7557-7568.
5. Wu C., (1995), Annu. Rev. Cell Dev. Biol., 11, 441-469.
6. Abravaya K., Myers M. P., Murphy S. P., Morimoto R. I., (1992), Genes Dev., 6, 1153-1164.
7. Craig E. A., Gross C. A., (1991), Trends Biochem. Sciences, 16, 135-140.
8. Mosser D. D., Duchaine J., Massie B., (1993), Moll. Cell. Biol., 13, 5427-5438.
9. Zuo J., Guo Y., Guettouche T., Smith D. F., Voellmy R., (1998), Cell, 94, 471-480.
10. Bruce J. L., Price B. D., Coleman C. N., Calderwood S. K., (1993), Cancer Res., 53, 12-15.
11. Jurivich D. A., Sistonen L., Kroes R. A., Morimoto R. I., (1992), Science, 255, 1243-1245.
12. Zuo J., Rungger D., Voellmy R., (1995), Mol. Cell. Biol., 15, 4319-4330.
13. Green M., Schuetz T. J., Sullivan E. K., Kingston R. E., (1995), Mol. Cell. Biol., 15, 3354-3362.
14. Shi Y., Kroeger P. E., Morimoto R. I., (1995), Mol. Cell. Biol., 15, 4309-4318.
15. Cotto J. J., Kline M., Morimoto R. I., (1996), J. Biol. Chem., 271, 3355-3358.
16. Xia W., Voellmy R., (1997), J. Biol. Chem., 272, 4094-4102.
17. Scharf K.-D., Rose S., Zott W., Schöffl F., Nover L., (1990), EMBO J., 9, 4495-4501.
18. Scharf K.-D., Rose S., Thierfelder J., Nover L., (1993), Plant Physiol., 102, 1355-1356.

19. Hübel A., Schöffl F., (1994), Plant Mol. Biol., 26, 353-363.
20. Czarnecka-Verner E., Yuan C.-X., Fox P. C., Gurley W. B., (1995), Plant Mol. Biol., 29, 37-51.
21. Gagliardi D., Breton C., Chaboud A., Vergne P., Dumas C., (1995), Plant Mol. Biol., 29, 841-856.
22. Bevan M., et al., (1998), Nature, 391, 485-488.
23. Prändl R., Hinderhofer K., Eggers-Schumacher G., (1998), Mol. Gen. Genet., 258, 269-278.
24. Nover L., Scharf K.-D., Gagliardi D., Vergne P., Czarnecka-Verner E., Gurley W. B., (1996), Cell Stress & Chaperones, 1, 215-223.
25. Boscheinen O., Lyck R., Queitsch C., Treuter E., Zimarino V., Scharf K.-D., (1997), Mol. Gen. Genet., 255, 322-331.
26. Czarnecka-Verner E., Yuan C.-X., Nover L., Scharf K.-D., Englich G., Gurley W. B., (1999), submitted.
27. Hübel A., Lee J. H., Wu C., Schöffl F., (1995), Mol. Gen. Genet., 248, 136-141.
28. Lee J. H., Hübel A., Schöffl F., (1995), Plant J., 8, 603-612.
29. Lee J. H., Schöffl F., (1996), Mol. Gen. Genet., 252, 11-19.
30. Damberger F. F., Pelton J. G., Harrison C. J., Nelson H. C. M., Wemmer D. E., (1994), Protein Sci., 3, 1806-1821.
31. Damberger F. F., Pelton J. G., Liu C., Chao H., Harrison C., Nelson H. C., Wemmer D. E., (1995), J. Mol. Biol., 254, 704-719.
32. Harrison C. J., Bohm A. A., Nelson H. C. M., (1994), Science, 263, 224-227.
33. Schultheiss J., Kunert O., Gase U., Scharf K. D., Nover L., Ruterjans H., (1996), Eur. J. Biochem., 236, 911-921.
34. Vuister G. W., Kim S. J., Wu C., Bax A., (1994), Biochemistry, 33, 10-16.
35. Fernandes M., Xiao H., Lis J. T., (1994), Nucleic Acids Res., 22, 167-173.
36. Kroeger P. E., Sarge K. D., Morimoto R. I., (1993), Mol. Cell. Biol., 13, 3370-3383.
37. Perisic O., Xiao H., Lis J. T., (1989), Cell, 59, 797-806.
38. Shuey D. J., Parker C. S., (1986), J. Biol. Chem., 261, 7934-7940.
39. Peteranderl R., Nelson H. C. M., (1992), Biochem., 31, 12272-12276.
40. Nakai A., Tanabe M., Kawazoe Y., Inazawa J., Morimoto R. I., Nagata K., (1997), Mol. Cell. Biol., 17, 469-481.
41. Kim S. J., Tsukiyama T., Lewis M. S., Wu C., (1994), Protein Sci., 3, 1040-1051.
42. Taylor I. C. A., Workman J. L., Schuetz T. J., Kingston R. E., (1991), Genes Dev., 5, 1285-1298.
43. Wu C., Wilson S., Walker B., Dawid I., Paisley T., Zimarino V., Ueda H., (1987), Science, 238, 1247-1253.
44. Nakai A., Morimoto R. I., (1993), Mol. Cell. Biol., 13, 1983-1997.
45. Czarnecka E., Fox P. C., Gurley W. B., (1990), Plant Physiol., 94, 935-943.
46. Nover L., Scharf K.-D., (1997), Cell. Mol. Life Sci., 53, 80-103.
47. Newton E. M., Knauf U., Green M., Kingston R. E., (1996), Mol. Cell. Biol., 16, 839-846.
48. Yuan C.-X., Czarnecka-Verner E., Gurley W. B., (1997), Cell Stress & Chaperones, 2, 263-275.
49. Wisniewski J., Orosz A., Allada R., Wu C., (1996), Nucleic Acids Res., 24, 367-374.
50. Treuter E., Nover L., Ohme K., Scharf K.-D., (1993), Mol. Gen. Genet., 240, 113-125.
51. Leuther K. K., Salmeron J. M., Johnston S. A., (1993), Cell, 72, 575-585.
52. Scharf K.-D., Materna T., Treuter E., Nover L., (1994), *Heat stress promoters and transcription factors*. in: *Plant Promoters and Transcription Factors*, Ed. L. Nover, Ed. Springer-Verlag, Berlin-Heidelberg, 121-158.
53. Nover L., Neumann D., Scharf K.-D., (1990), *Heat shock and other stress response systems of plants*, in: *Results and problems in cell differentiation*, Ed. K.-D. Scharf, (Springer-Verlag, Berlin, Germany, vol. 16.
54. Scharf K.-D., Heider H., Hoehfeld I., Lyck R., Schmidt E., Nover L., (1998), Mol. Cell. Biol., 18, 2240-2251.
55. Lyck R., Harmening U., Hohfeld I., Treuter E., Scharf K. D., Nover L., (1997), Planta, 202, 117-125.

56. Cotto J. J., Fox S. G., Morimoto R. I., (1997), J. Cell Sci., 110, 2925-2934.
57. He B., Meng Y.-H., Mivechi N. F., (1998), Mol. Cell. Biol., 18, 6624-6633.
58. Jolly C., Morimoto R. I., Robert-Nicoud M., Vourc'h C., (1997), J. Cell Sci., 110, 2935-2941.
59. Czarnecka-Verner E., Yuan C.-X., Nover L., Scharf K.-D., Englich G., Gurley W. B., (1997), Acta Physiol. Plant., 19, 529-537.
60. Kroeger P. E., Morimoto R. I., (1994), Mol. Cell. Biol., 14, 7592-7603.
61. Shimizu S., Itoh Y., Yamazaki K., (1996), Plant Mol. Biol., 31, 13-22.

Plant heat shock transcription factors: divergence in structure and function

Summary

A multitude of heat shock transcription factors (HSFs) have been isolated and characterized from various plant species (17-23). Based on a phylogeny analysis of the DNA binding domains and organization of oligomerization domains, they have been assigned to class A and B of the plant HSF family (20,24 and this paper). None of the tested soybean or *Arabidopsis* HSF class B members were able to function as transcriptional activators and are, therefore, considered to be inert (26,59). Conversely, class A HSFs from tomato and *Arabidopsis* displayed an intrinsic transcriptional activation potential (26,50). There seems to be variation among plant class A HSFs regarding their transcriptional activation functions: some play a key role in activation of the heat shock response, while others act in an auxiliary capacity as HSF activity boosters (54). In contrast, the class B inert HSFs are able to *trans*-attenuate the transcriptional activity of activator HSFs (26). We postulated that heat shock regulation in plants may differ from metazoans by partitioning negative and positive functional domains onto separate HSF proteins (59). In plants two classes of HSFs exist: class A members which function as activators of HSP gene expression, and a novel class B (inert HSFs) which is largely specialized for repression, or attenuation, of the heat shock response.

Key words:
transcription regulation, heat shock transcription factor, activation domain.

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