

INVITED REVIEW

Could Heat Shock Transcription Factors Function as Hydrogen Peroxide Sensors in Plants?

GAD MILLER and RON MITTLER*

Department of Biochemistry and Molecular Biology, University of Nevada, Mail Stop 200, Reno, NV 89557, USA

Received: 21 December 2005 Returned for revision: 6 February 2006 Accepted: 2 March 2006 Published electronically: 1 June 2006

• *Background* Heat shock transcription factors (Hsfs) are modular transcription factors encoded by a large gene family in plants. They bind to the consensus sequence 'nGAAnnTCCn' found in the promoters of many defence genes, and are thought to function as a highly redundant and flexible gene network that controls the response of plants to different environmental stress conditions, including biotic and abiotic stresses. Hsf proteins encoded by different genes exhibit a high degree of complexity in their interactions. They can potentially bind and activate their own promoters, as well as the promoters of other members of their gene family, and they can form homo- or heterotrimers resulting in altered nuclear localization, as well as enhanced or suppressed transcription.

• *Scope* In this review, we summarize recent studies on Hsf function in *Arabidopsis* and tomato and present evidence obtained from microarray expression studies in *Arabidopsis* that the Hsf gene network is highly flexible and specialized, with specific members and/or member combinations controlling the response of plants to particular stress conditions. In addition, we describe recent studies that support the hypothesis that certain Hsfs function as molecular sensors that directly sense reactive oxygen species (ROS) and control the expression of oxidative stress response genes during oxidative stress.

Key words: Heat shock transcription factors (Hsfs), reactive oxygen species (ROS), abiotic stress, gene network, sensor.

INTRODUCTION

The heat shock (HS) response is a highly conserved response, characterized by rapid induction of heat shock proteins (HSPs) that primarily function as molecular chaperones to ensure the correct function of many cellular proteins under conditions of elevated temperature. The HS response was shown to be controlled by heat shock transcription factors (Hsfs) that act by binding to the highly conserved heat shock element (HSE; a palindromic motif of nGAAn) in the promoters of target genes. A key step in the activation process of Hsfs, in response to different stress conditions, involves the formation of homotrimers with high affinity for the HSE. In addition to mediating a relatively large part of the defence response of eukaryotes to heat stress, Hsfs are also thought to be involved in different pathological conditions, cellular responses to oxidative stress, heavy metals, amino acid analogues and metabolic inhibitors, and certain developmental and differentiation processes (Sorger and Pelham, 1988; Park and Craig, 1989; Jedlicka *et al.*, 1997; Morimoto, 1998; Hahn *et al.*, 2004).

An intimate relationship appears to exist between oxidative stress and the HS response (Liu and Thiele, 1996; McDuffe *et al.*, 1997; Ahn and Thiele, 2003). When the HS response was first identified in *Drosophila* by Ritossa *et al.* in 1962, it was also shown to be induced during recovery from anoxia, which results in oxidative stress. Heat stress was shown to cause impairments in mitochondrial functions that result in the induction of oxidative damage (Davidson and Schiestl, 2001; Larkindale and Knight 2002; Vacca *et al.*, 2004). In plants, the steady-state transcript and protein level of

many reactive oxygen species (ROS)-scavenging enzymes was found to be elevated by heat stress (Rainwater *et al.*, 1996; Sato *et al.*, 2001; Rizhsky *et al.*, 2002; Mittler *et al.*, 2004; Vacca *et al.*, 2004). In addition, acquired thermotolerance, i.e. the ability of plants to develop heat tolerance following a mild heat pre-treatment, was shown to be mediated in part by enhancing cellular mechanisms that prevented oxidative damage under heat stress (Bergmüller *et al.*, 2003; Larkindale and Huang, 2004). HS leading to programmed cell death in plants was also shown to be associated with an enhanced production of ROS and the activation of the oxidative burst (Vacca *et al.*, 2004). The intimate relationship between the HS and oxidative stress responses, the activation of Hsfs during these processes, and recent genetic and biochemical studies (described below) suggest that Hsfs might function as direct sensors of hydrogen peroxide in plants. In this review, we will summarize evidence supporting this hypothesis, as well as propose a model for the function of the Hsf network in plants.

EUKARYOTIC HSF GENES

Yeast and *Drosophila* contain only one Hsf gene, while vertebrates have four Hsfs. In contrast, plants show a much higher complexity, with Hsf genes comprising whole networks of approx. 18 (tomato) to 34 (soybean) Hsf genes (Nover *et al.*, 1996, 2001; Kotak *et al.*, 2004). The yeast Hsf protein was shown to be vital for survival. It constitutively binds the HSE and displays a basal level of transcriptional activation required under normal conditions. The yeast Hsf gene is therefore involved in many aspects of cell functioning such as protein

* For correspondence. E-mail ronm@unr.edu

degradation, carbohydrate metabolism and energy generation (Sorger and Pelham, 1988; Yamamoto *et al.*, 2005). In contrast to yeast, *Drosophila* Hsf is required for early developmental stages, but is dispensable for general cell growth and viability (Jedlicka *et al.*, 1997). In vertebrates, that have four Hsf proteins, Hsf1 is the primary Hsf activated during HS (Sarge *et al.*, 1993). It is, however, not essential for survival under normal conditions. In addition to Hsf1, Hsf3 is also inducible during HS. Interestingly, the four vertebrate Hsfs show diverse regulatory responses to a wide spectrum of environmental and developmental signals, demonstrating a high degree of complexity exhibited by a relatively small gene family (Rabindran *et al.*, 1991; Sarge *et al.*, 1991; Schuetz *et al.*, 1991; Nakai and Morimoto, 1993; Nakai *et al.*, 1997; Morimoto, 1998; Tanabe *et al.*, 1998).

The model plant *Arabidopsis thaliana* contains 21 Hsf genes, as well as several genes encoding Hsf-like proteins. More than 16 Hsf genes are found in tomato, and many other Hsf genes were identified in rice, maize and other species. Plant Hsf genes were assigned to three different classes (classes A, B and C) according to their unique structural characteristics (Nover *et al.*, 2001). Class A HSF proteins comprise the largest group of Hsfs with 15 proteins in *Arabidopsis*. They contain an activation domain at the C-terminus and are thought to be involved in transcriptional activation. Class B and class C Hsfs lack a defined aromatic/hydrophobic/acidic (AHA)-type activation domain. The absence of an activation domain, as well as their inability to rescue the yeast Hsf1 mutation, has led to the assumption that class B Hsfs function as repressors (Boscheinen *et al.*, 1997; Czarnecka-Verner *et al.*, 2000, 2004). However, HsfB1 was recently demonstrated to function as a novel co-regulator of the tomato HsfA1 or HsfA2 enhancing their transcriptional activity (Bharti *et al.*, 2004).

HSF STRUCTURE

Hsfs have a modular structure, conserved among eukaryotes (Fig. 1). All Hsfs contain a DNA-binding domain (DBD), an oligomerization domain, a nuclear localization sequence (NLS) and in most cases a nuclear export sequence (NES) (Nover *et al.*, 1996, 2001). The DBD is the most conserved domain among eukaryotic Hsfs. It contains a helix–turn–helix motif (H2–T–H3), that allows the specific recognition and binding of the palindromic HSEs (Damberger *et al.*, 1994; Harrison *et al.*, 1994; Vuister *et al.*, 1994; Schulthess *et al.*, 1996). The DBD of all plant Hsf genes contains an intron, located immediately downstream of the H2–T–H3 motif (Nover *et al.*, 2001). Downstream of the DBD and separated by a flexible linker peptide which is variable in size, two adjacent hydrophobic heptad repeats (HR-A and HR-B) comprise the oligomerization domain. It is thought that the primary role of the HR-A/B is to provide hydrophobic surfaces for Hsf trimerization (Zuo *et al.*, 1994).

The three classes of plant Hsf genes were defined based on peculiarities in the flexible linker and the HR-A/B domains (Nover *et al.*, 2001). Class A, that comprise the largest group, contain a C-terminal activation domain (CTAD), that is the least conserved in sequence and size (Nover *et al.*, 2001). The function of the class A Hsfs as transcriptional activators depends on a short AHA motif found in the CTAD, enriched with aromatic, large hydrophobic and acidic amino acids (Nover and Scharf, 1997; Doring *et al.*, 2000). AHA domains are crucial for the interaction of Hsfs with the transcriptional machinery, as was shown by pull-down experiments and functional analysis of class A Hsfs (Yuan and Gurley, 2000; Kotak *et al.*, 2004). Another hydrophobic repeat (HR-C) is found at the C-terminal domain (Nover *et al.*, 2001). This region is conserved among animal Hsfs but it is poorly conserved in yeast and plant Hsfs (Wu, 1995). The NES is a leucine-rich export signal found at the C-terminus of the CTAD. The subcellular distribution of Hsfs is dictated by the balance between nuclear import and nuclear export as determined by the relative strengths of the NLS and NES, and possibly by intermolecular interactions between different Hsf monomers (Gorlich and Kutay, 1999; Heerklotz *et al.*, 2001). In support of this model, disruption of the C-terminal domain of *Arabidopsis* Hsfs restricts Hsf proteins to the nucleus (Kotak *et al.*, 2004). In contrast to plant Hsfs, the C-terminal HR-C domain of animals is highly conserved (Wu *et al.*, 1995). Under basal conditions, the human Hsf1, as well as the *Drosophila* Hsf, are maintained as inactive monomers via intramolecular interactions between HR-C and HR-A/B, suppressing trimer formation (Rabindran *et al.*, 1993; Zuo *et al.*, 1994). This type of intramolecular suppression is thought not to exist in plants Hsfs.

THE PLANT HSF NETWORK

The number of plant Hsfs continues to grow. A new signature domain of the CTAD containing the AHA and NES motifs of plants allowed the identification of >60 new class A Hsfs from expressed sequence tag (EST) databases, including 19 new Hsfs in soybean (34 in total) and at least 23 Hsfs in rice (Kotak *et al.*, 2004). The complexity of the plant Hsf gene family is thought to allow a highly flexible and efficient response to rapid changes in environmental conditions that accompany the stationary lifestyle of plants (Nover *et al.*, 2001; Kotak *et al.*, 2004). Figure 2, generated from Genevestigator microarray data sets (Zimmermann *et al.*, 2004; <https://www.genevestigator.ethz.ch/>), reveals a surprisingly diverse basal level of expression of the *Arabidopsis* Hsf gene network in different plant tissues. Previously, transcripts encoding Hsfs A3, A6a, A6b, A7b, B2a and B3 could not be detected using RNA from control or heat-stressed tissue-cultured cells or leaves (Nover *et al.*, 2001). However, as shown in Fig. 2, apart from HsfA6a that was not expressed in all tissues, all other Hsf genes demonstrated a low to moderate level of expression in most tissues, or in a tissue-specific manner.

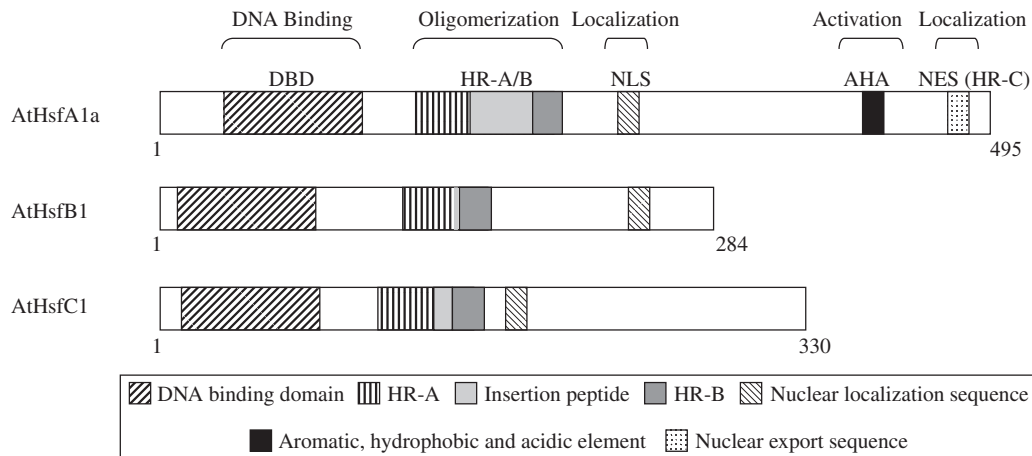


FIG. 1. *Arabidopsis* Hsf protein structure. A model representing the structure of *Arabidopsis* HsfA1a (A-Class Hsf), HsfB1 (B-Class Hsf) and HsfC1 (C-Class Hsf) is shown. The different domains are indicated on the top and different structural features present in each domain are outlined below. The models presented in the figure were generated according to Nover *et al.* (2001).

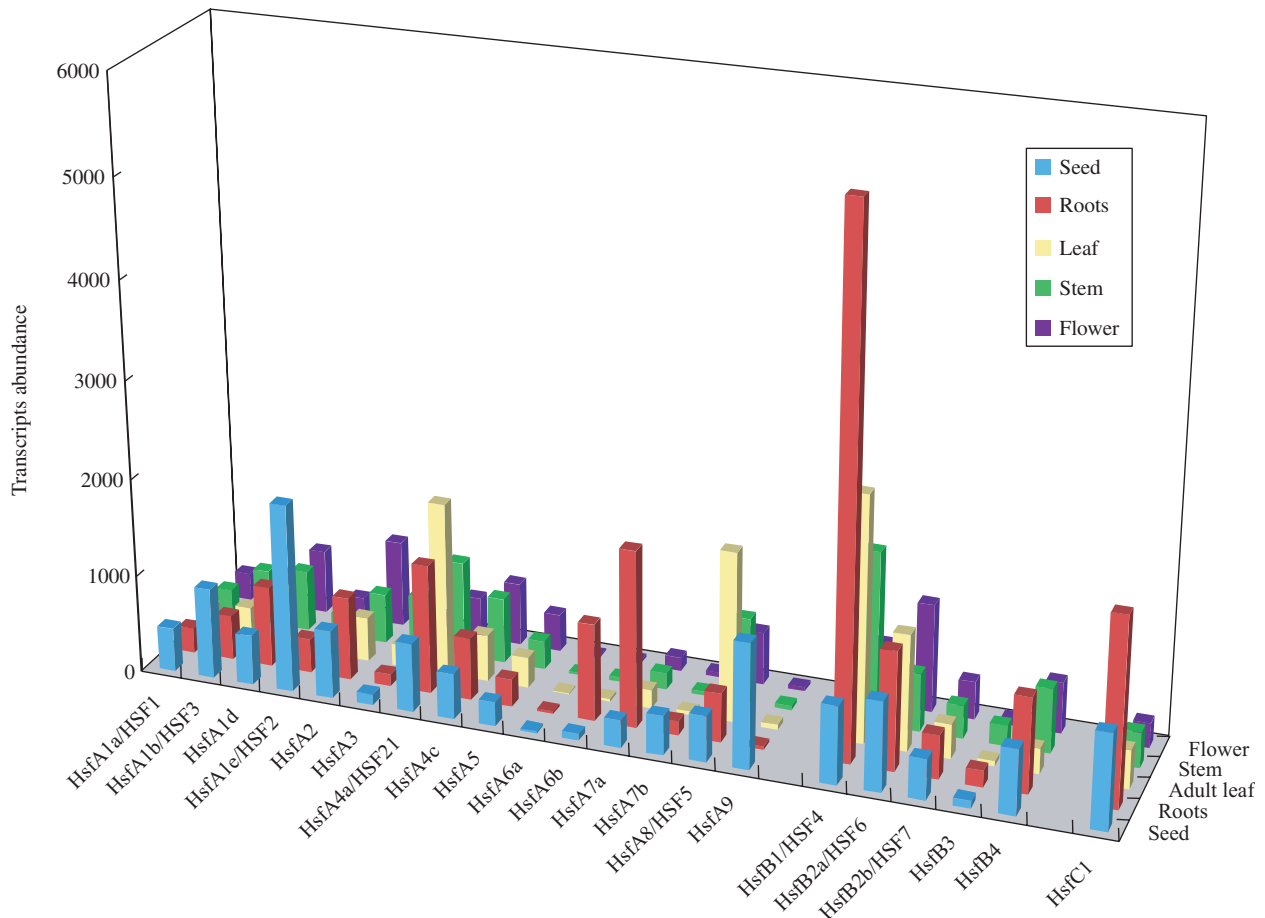


FIG. 2. Steady-state transcript level of *Arabidopsis* HSFs in different tissues. The basal steady-state transcript level of all *Arabidopsis* Hsfs was obtained from the Genevestigator microarray database using the 'Meta-analyzer' tool (Zimmermann *et al.*, 2004). The values represent the signal intensity of each probe set as given by Genevestigator (<https://www.genevestigator.ethz.ch/>).

The best-studied plant Hsf system is that of tomato (*Lycopersicon peruvianum*). The tomato HsfA1, a constitutively expressed Hsf, is the master regulator of the heat response and is essential for thermotolerance

(Mishra *et al.*, 2002; Baniwal *et al.*, 2004). Plants with suppressed LpHsfA1 do not survive a moderate heat stress (1 h at 45 °C), suggesting that the function of LpHsfA1 could not be compensated for by other LpHsfs. In

contrast, enhanced expression of LpHsfA1 in transgenic plants enhances thermotolerance, even to severe heat stress conditions (Mishra *et al.*, 2002; Baniwal *et al.*, 2004). The tomato HsfA2 is considered the ‘work horse’ of the HS response in tomatoes. It accumulates in cells after heat stress induction and becomes the dominant Hsf of cells. The synthesis of LpHsfA2 is controlled by the activation of LpHsfA1 (Mishra *et al.*, 2002; Baniwal *et al.*, 2004). LpHsfA2 is found in cells in three different forms: (1) a soluble cytoplasmic form in heat-acclimated cells; (2) a nuclear form found in HS-treated cells; and (3) a stored form in cytoplasmic HS granules (HSGs), which represents a major site for HSP accumulation (Nover *et al.*, 1983, 1989). Hsp17.4-CII was found to interact with the C-terminal domain of LpHsfA2 and to act as a co-repressor forming aggregates that can be solubilized in the presence of class CI sHsps, or by hetero-oligomerization with LpHsfA1 (Port *et al.*, 2004). The tomato HS-induced HsfA2 is dependent on LpHsfA1 expression for nuclear localization and for its nuclear retention by formation of an LpHsfA1–LpHsfA2 hetero-oligomer (Scharf *et al.*, 1998; Heerklotz *et al.*, 2001; Port *et al.*, 2004). In contrast, *Arabidopsis* HsfA1a is not required for the HS-dependent expression of AtHsfA2 (Busch *et al.*, 2005), nor for its nuclear localization (Kotak *et al.*, 2004).

In *Arabidopsis*, in contrast to tomato, no AtHsf master regulator could be identified. The *Arabidopsis* loss-of-function mutants *AthsfA1a* and *AthsfA1b* alone had no obvious effects on the HS response, only the *AthsfA1a/1b* double mutant was impaired in HS gene expression, showing lower transcript levels of HSPs at early stages of the HS response (Lohmann *et al.*, 2004; Busch *et al.*, 2005). The lack of a strong negative effect of the double mutant might suggest that in *Arabidopsis* other Hsf proteins can compensate for AtHsfA1a and AtHsfA1b; however, there was no increase in the expression of any of the other 13 class A Hsf genes in the *AtHsfA1a/1b* double mutant. These results show that AtHsfA1a and AtHsfA1b are necessary for early onset of HS gene expression at the transcriptional level, but they are not the sole regulators of the HS response in *Arabidopsis* (Lohmann *et al.*, 2004). Only a small fraction (4%) of the HS-regulated genes were associated with AtHsfA1a/1b function (Busch *et al.*, 2005).

Figure 3 summarizes changes in the steady-state transcript level of different members of the *Arabidopsis* Hsf gene family in leaves of plants subjected to different abiotic stresses. The data presented in Fig. 3 reveal a high diversity in the response of different AtHsf genes to different abiotic stresses. Based on the data presented in Fig. 3, it is suggested that there is a high degree of specialization in the response of specific Hsfs to particular stress conditions. Thus, for example, AtHsfA9 appears to be specific to salt, drought and cold stress, while AtHsfA6a and AtHsfA6b appear to be cold and salt specific. With the exception of AtHsfA2 and AtHsfB1, the pattern of Hsf expression during heat stress was different from the pattern of Hsf expression during other stresses. Because HSEs are found in the promoters of many defence genes (e.g. Rizhsky *et al.*, 2004a), it is possible

that different Hsfs, expressed during different stresses, activate or control different defence pathways. When the HSP response of plants subjected to drought, heat or drought combined with heat was compared, for example, it was found that all HSPs expressed during heat were also expressed during drought combined with heat and/or drought. However, leaves subjected to drought or drought combined with heat expressed specific HSPs not found in heat stress-treated leaves (Rizhsky *et al.*, 2004b). The combinatorial function of Hsfs could therefore be responsible for stress-specific expression of HSPs or other defence genes, and specific stress conditions could therefore cause activation of a particular set(s) of different Hsfs (Fig. 3; Rizhsky *et al.*, 2004b). Because most of the experiments presented in Fig. 3 include only a limited number of time points, the complete expression pattern of the Hsf network during different stress responses might not be captured by the figure. The data presented in Fig. 3 suggest that in addition to being potentially redundant, the Hsf gene network is highly flexible and specialized. It controls the response of plants to diverse stress conditions, as well as potentially their combination (Rizhsky *et al.*, 2004b; Mittler, 2006).

The promoters of all AtHsf genes contain clusters of HSEs (Nover *et al.*, 2001), suggesting that the expression of this transcription factor gene family could be self-regulated. Potentially, each AtHsf could bind to the HSEs of every AtHsf, including its own, and activate or repress its expression, although there is still no evidence to support such self-regulation. In addition, approx. 33% of 22 810 *Arabidopsis* genes analysed were found to contain the consensus motif (nGAAnnTCCn) of HSE within 1000 bp of their putative promoter sequence (Busch *et al.*, 2005). This may suggest that there are other requirements for Hsf-mediated gene activation/suppression, since it is hard to expect that every gene’s promoter containing HSE is regulated by Hsfs.

Functional interdependence studies between Hsfs, co-immunoprecipitation and yeast one-hybrid assays suggest that all class A LpHsfs can interact with each other, potentially forming hetero-oligomers (Scharf *et al.*, 1998; Bharti *et al.*, 2000). For example, the LpHsfA3 was isolated from a heat stress cDNA library by a two-hybrid screen using LpHsfA1 as a bait (Bharti *et al.*, 2000). Furthermore, different Hsfs can associate with each other potentially functioning as co-activators or co-repressors. According to an as yet unpublished observation reported by Baniwal *et al.* (2004), the tomato and *Arabidopsis* HsfA4a specifically interact with AtHsfA5 and function as co-repressors. In addition, the activity of AtHsfA4a was strongly repressed when co-expressed with AtHsfB1 (Czarnecka-Verner *et al.*, 2000). AtHsfB1 was later shown to repress the transcriptional activity of class A Hsfs through an active mechanism that involves its C-terminal regulatory region (Czarnecka-Verner *et al.*, 2004). In contrast, the tomato LpHsfB1 acts as a co-activator of class A LpHsfs. The co-activation of A-type LpHsfs by LpHsfB1 depends on a histone-fold-like motif in its C-terminal domain, which is required for the recruitment of the plant CREB-binding protein orthologue

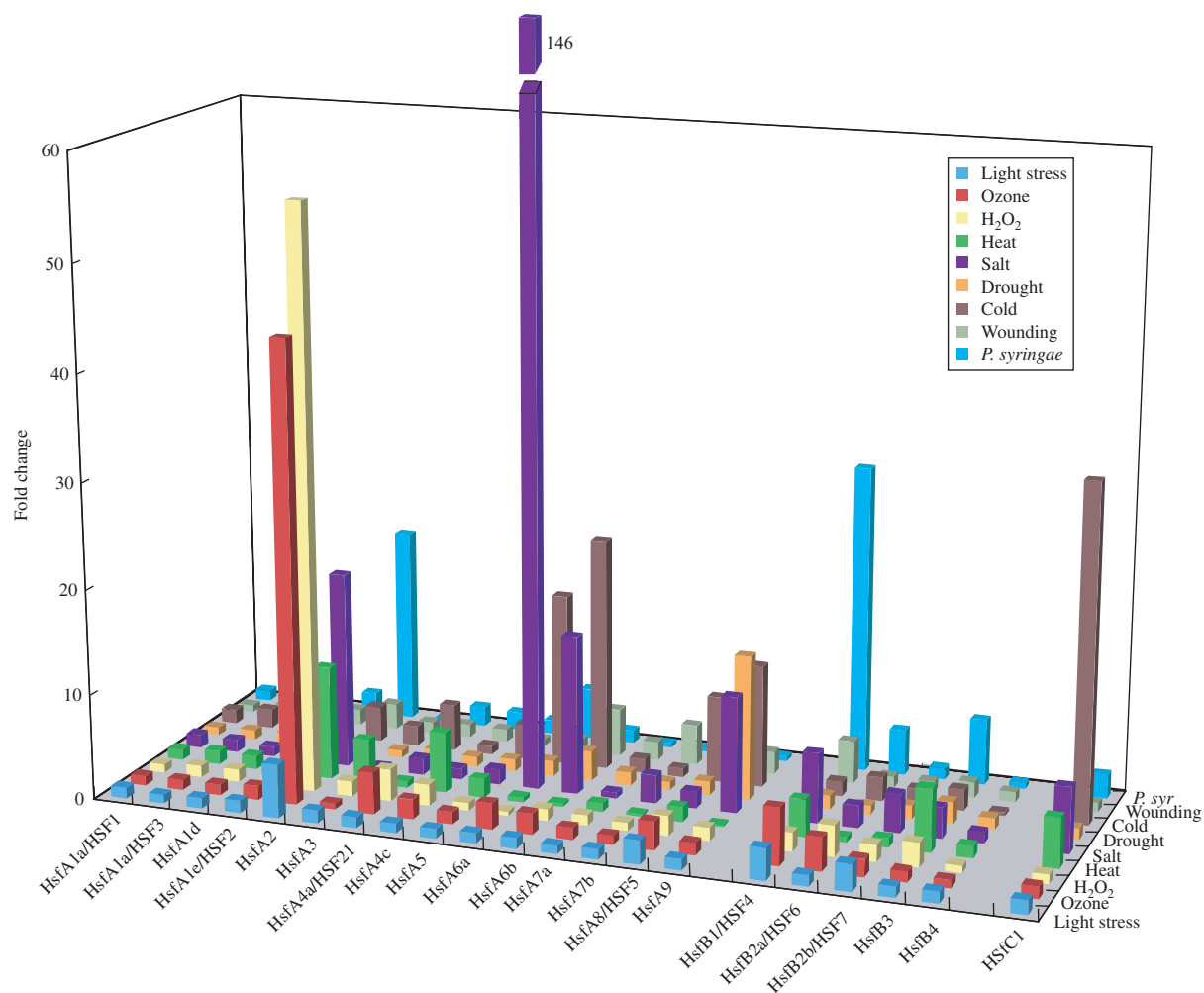


FIG. 3. *Arabidopsis* Hsf transcript accumulation in response to different environmental stress conditions. The values are presented as fold change, relative to control treatment or $t = 0$ min of the experiment. All data, except for the light stress experiment, were obtained from the Genevestigator microarray database using the 'digital-northern' tool (Zimmermann *et al.*, 2004). The relative induction levels of Hsfs during moderate light stress were obtained from a microarray experiment previously described by Davletova *et al.* (2005a). To demonstrate maximal fold increase for each Hsf in each stress treatment, in each experiment that contained several time points, the time point in which a particular Hsf showed the highest expression level was chosen for representation. In general, the following time points were used: light stress (30 min), salt (12 h), drought (24 h), cold (24 h) and wounding (24 h). The following exceptions were made for particular Hsfs showing the highest level of induction at other time points: Light stress: HsfB1, 3 h; HsfA8, 6 h. Salt stress: HsfA1a, HsfA4a, HsfA6a, HsfB2a, 6 h; A1e, A8, A9, B1, B2b and B3, 24 h. Cold stress: A4a and B2a, 6 h; A1d and A5, 12 h. Wounding: B1 and 6a, 6 h.

HAC1. The stimulation effect of LpHsfB1 was not restricted only to interaction with LpHsfA1, but could also be observed with LpHsfA2 (Bharti *et al.*, 2004). AtHsfB1 on the other hand, lacking the crucial lysine residue in the histone-fold motif, does not function as a co-activator and potentially even interferes with the activation of class A Hsfs via competition for HSE binding (Bharti *et al.*, 2004).

The complexity of the Hsf gene network of plants is evident at at least five different levels: (1) a large number of Hsf genes are present in the plant genome; (2) each Hsf gene can potentially bind to its own promoter, as well as to the promoters of all other Hsf genes; (3) monomers encoded by different Hsf genes can interact leading to activation or suppression of transcription; (4) monomers encoded by different Hsf genes can interact affecting nuclear targeting and retention; and (5) spatial and temporal expression

patterns of Hsfs could affect different responses in different tissues. These features make the Hsf gene network a highly redundant and specialized network that functions in a stress- or developmental-specific manner.

REDOX REGULATION OF HSFS IN EUKARYOTES

Considerable evidence supporting a possible role for plant Hsfs as direct sensors of ROS can be found in studies of mammalian, *Drosophila* and yeast Hsfs. In general, the transcriptional activation of Hsfs is achieved in two stages. First, inactive Hsf monomers form a homo-trimer upon receiving a stress signal. The DNA-binding trimer can then be modified further by phosphorylation, or subjected to conformational changes that activate transcription (Fig. 4; Larson *et al.*, 1988; Lee *et al.*, 2000).

Human Hsf1 and *Drosophila* Hsf were shown to sense hydrogen peroxide directly and assemble into a homotrimer in a reversible and redox-regulated manner (Zhong *et al.*, 1998; Ahn and Thiele, 2003). It was demonstrated that two cysteine residues, located within and near the DBD, are required for intramolecular disulfide bond formation in response to heat or H₂O₂ stress. The conformational change induced by the disulfide formation was shown in turn to be essential for the formation of HsHsf1 homotrimers, nuclear translocation and gene activation (Hahn and Thiele, 2004). H₂O₂ was also found to induce transactivation, nuclear translocation and DNA binding activity of *Drosophila* Hsf1 (Jacquier-Sarlin and Polla, 1996). Furthermore, the DNA binding of the *Drosophila* Hsf1 protein was shown to be reversibly regulated by H₂O₂ as well as by high temperature (Fig. 4; Zhong *et al.*, 1998).

In yeast, superoxide anions (O₂⁻) directly induced a conformational change in the Hsf DNA-binding trimer from its low-activity mode to a high-activity mode. The O₂⁻ signal was shown to be perceived by the region that links the DBD and the trimerization domains of the yeast Hsf, promoting the physical interaction of two DNA-bound homotrimers (Lee *et al.*, 2000). The yeast Hsf is required under oxidative stress for the activation of Cu, Zn superoxide dismutase (SOD), presenting a direct circle of regulation in which O₂⁻ activates the yeast Hsf that induces CuZnSOD which scavenges O₂⁻ and suppresses the activation of Hsf (Liu and Thiele, 1996). In yeast, Skn7, a response regulator that contains a two-component system receiver domain, was shown to be required for the activation of HS gene expression specifically in response to hydrogen peroxide. Skn7 was found to bind to HSEs and physically associate with Hsf1, functioning as an essential co-activator during oxidative stress conditions (Raitt *et al.*, 2000). Interestingly, a familial form of amyotrophic lateral sclerosis (ALS) neurodegenerative disease was shown to be associated with autosomal dominant mutations of Cu/ZnSOD1, which accumulate in a non-native conformation and aggregate. An HsHsf1 dominant positive mutant was shown to protect neurons from mutant Cu/ZnSOD-1 toxicity by stimulating Hsp70 expression (Bruening *et al.*, 1995; Batulan *et al.*, 2003). Thus, it was suggested that the sensitivity of motor neurons to stress could result from maladjusted mechanisms of stress activation of Hsf1 (Voellmy, 2004).

HSFS AND OXIDATIVE STRESS IN PLANTS

The promoter of the central H₂O₂-scavenging enzyme cytosolic ascorbate peroxidase 1 (Apx1), as well as the promoters of many defence genes and transcription factors involved in H₂O₂ signalling and defence, contain an Hsf-binding motif (Mittler and Zilinskas, 1992; Rizhsky *et al.*, 2004a; Davletova *et al.*, 2005a). Promoter analyses, as well as overexpression studies of AtHsfA1b in *Arabidopsis*, suggest that the Hsf binding site at the Apx1 promoter is functional (Storozhenko *et al.*, 1998; Panchuk *et al.*,

2002). Furthermore, several other *Arabidopsis* Apx genes showed enhanced transcript accumulation in response to a short-term heat shock in an AtHsfA1b-dependent mechanism (Panchuk *et al.*, 2002). The activity of *Arabidopsis* Apx2 is specifically induced by high light stress and high temperature. Apx2 was also shown to be AtHsfA1b dependent (Panchuk *et al.*, 2002). This result coincides with the observation of Lohmann *et al.* (2004, data were not provided) of a strong negative effect on the induction of Apx2 in the *Athsfa1a/1b* double mutant.

ROS such as H₂O₂, O₂⁻ and ¹O₂ are thought to function as early signals for high light stress in plants (Pnueli *et al.*, 2003; Rizhsky *et al.*, 2003; Apel and Hirt, 2004; Mittler *et al.*, 2004; Davletova *et al.*, 2005a). Accordingly, *Arabidopsis* mutants deficient in Apx1 accumulate higher levels of H₂O₂ compared with wild-type plants during light stress (Pnueli *et al.*, 2003; Davletova *et al.*, 2005a). Figure 5 compares the change in steady-state transcript level of all *Arabidopsis* Hsfs in wild-type and in knockout Apx1 (KO-Apx1) plants during light stress. The most dramatic difference was observed for AtHsfA4a and AtHsfA8 transcripts showing a constant higher level of expression in the Apx1 mutant (Davletova *et al.*, 2005a), while the expression pattern of other AtHsfs was only slightly affected or remained essentially unchanged in the absence of the Apx1 gene. The Hsfs whose transcripts transiently peaked during light stress in wild type as well as in the KO-Apx1 mutant are AtHsfs A2, A4a, A8, B1, B2b and C1 (Fig. 5). Of these, only AtHsfs A2, A4a, A8 and B1 showed elevated levels, ≥2-fold, during oxidative stress conditions, i.e. H₂O₂ or ozone or both (Fig. 3). HsfA2 increases approx. 50-fold under oxidative stresses, which suggest that its function might be important under these conditions; however, it did not increase in KO-Apx1 above its expression level in the wild type, suggesting that different AtHsfs might also respond differently to different types of oxidative stress, or to different levels of ROS. It could also be that the high level of expression of AtHsfA2 during oxidative stress conditions requires a transcriptional activator that is absent during light stress.

Thirty-two percent of the transcripts elevated in KO-Apx1 plants in response to a moderate light stress were also elevated in wild-type plants in response to H₂O₂ application (Davletova *et al.*, 2005b). HsfA4a and Zat12, as well as other genes that might be associated with H₂O₂ signalling, including NADPH oxidase (RbohD), MAPK3 and several WRKY transcription factors, were elevated under both conditions (Davletova *et al.*, 2005b). The zinc finger protein Zat12 is required for the expression of Apx1 during oxidative stress (Rizhsky *et al.*, 2004a; Davletova *et al.*, 2005b). Interestingly, a dominant negative construct for AtHsfA4a, when expressed in *Arabidopsis*, suppressed the expression of Zat12 and Apx1 during light stress (Davletova *et al.*, 2005a). This finding suggests that AtHsfA4a functions upstream of Zat12 and Apx1 (both containing an HSE element in their promoters; Rizhsky *et al.*, 2004a). Its rapid response to hydrogen peroxide stress, its control of Apx1 and Zat12 expression and its constitutive expression in cells in the absence of stress makes AtHsfA4a a prominent candidate to function as an

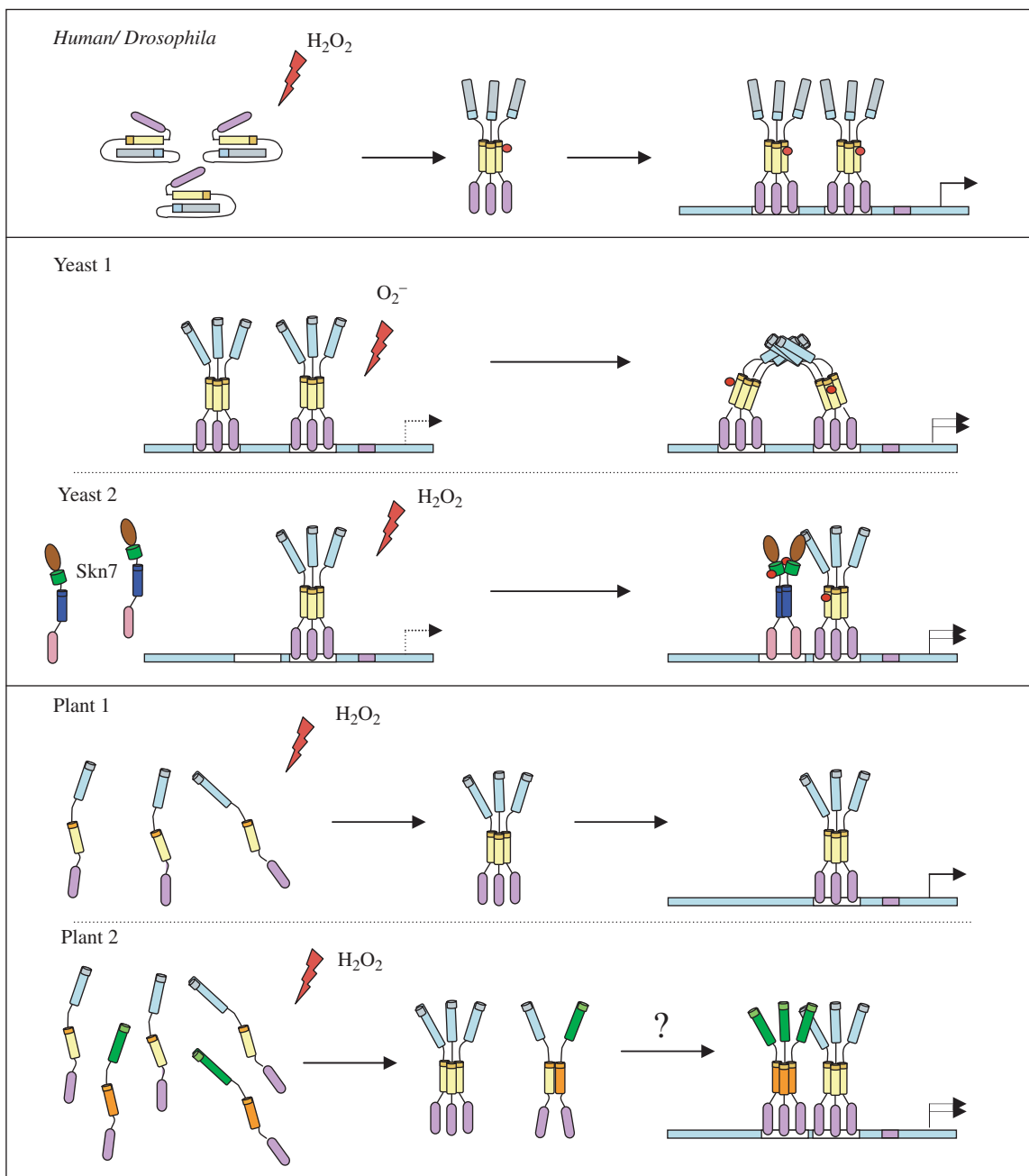


FIG. 4. A putative model for Hsf function in different organisms. The human and *Drosophila* Hsfs (upper panel) are inactive under non-stress conditions due to intramolecular interactions between HR-A/B and the HR-C domains. In response to hydrogen peroxide, the protein forms a disulfide bond between two cysteine residues inside and near the DNA-binding domain. The active Hsf is then phosphorylated and forms a transcriptionally active homotrimer that is transported to the nucleus and activates ROS-responsive gene expression. Two different target models are presented for oxidative stress-mediated activation of Hsf in yeast (middle panels). In the upper section, both Hsfs are bound to the HSEs of the target gene promoter as a homotrimer; superoxide anions directly interact with the protein inducing a conformational change that forms a cooperative interaction between them that increases their transcriptional activity. The second yeast model (lower section) shows the increase of Hsf activity by the co-activation of Skn7. Upon perception of H₂O₂ stress, Skn7, which is localized in the nucleus, is phosphorylated at the receiver domain by a histidine kinase sensor inducing the formation of an Skn7 homodimer. The active homodimer can bind to HSE adjacent to Hsf and increase its transcriptional activation. Two hypothetical models for plants are presented in the lower panels. A simplistic model is shown in the upper section in which Hsf (AtHsfA4a or AtHsfA2a) forms a homotrimer in response to interaction with ROS and is transported to the nucleus to activate oxidative stress gene expression. In the lower section, oxidative stress induces the homo-trimerization of a particular Hsf, which in response interacts with another Hsf to mediate its transport to the nucleus. The two active Hsfs can cooperatively induce gene expression. For example, AtHsfA8a could act as the co-activator. AtHsfA8a is expressed during oxidative stresses (Fig. 3), and in KO-Apx1 plants (see Fig. 5). It is localized to the cytoplasm and it could be dependent on other Hsfs for nuclear localization. Alternatively, the homotrimer of AtHsfA4a can be transported to the nucleus where it can cooperate with a class B Hsf (AtHsfB2b) or another class A Hsf.

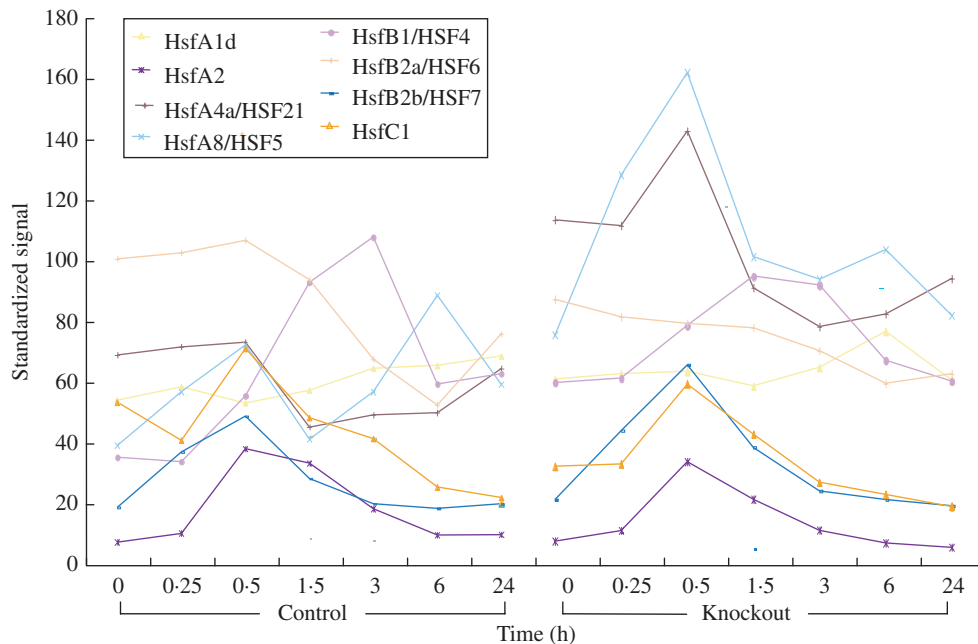


FIG. 5. Time course analysis of *Arabidopsis* Hsf accumulation in response to light stress in wild-type and knockout plants lacking the ROS defence enzyme cytosolic ascorbate peroxidase (KO-Apx1). The expression of the eight light stress-responsive Hsfs is presented, in the wild type (left) and KO-Apx1 (right) during time course exposure to moderate light stress. The steady-state transcripts level was obtained from microarray experiments previously described by Daveltova *et al.* (2005a).

Hsf H₂O₂ sensor in *Arabidopsis*. Furthermore, AtHsfA4a as well as AtHsfs A1a, A1b, A1d, A1e and A2 are evenly distributed in the cytoplasm and nucleus (Kotak *et al.*, 2004); this characteristic is highly important for the function of a sensor to detect changes in the cytosol and affect expression of the appropriate gene in the nuclei. In rice, HsfA4a is encoded by the *Spl7* gene. A point mutation in *Spl7* (tryptophan to arginine substitution in the DBD) caused a ‘disease lesion mimics’ phenotype, which suggests a role for HsfA4a as an anti-apoptotic factor (Yamanouchi *et al.*, 2002). Overproduction of H₂O₂ and O₂⁻ is known to be associated with programmed cell death, causing lesion formation (Mittler *et al.*, 1996; Torres *et al.*, 2002; Vacca *et al.*, 2004), supporting the hypothesis that HsfA4a is a redox-sensitive hydrogen peroxide sensor in plants. Nevertheless, based on the microarray results presented in Fig. 5, HsfA8 might also function as a potential H₂O₂ sensor. Further work is of course required to determine whether Hsfs such as HsfA4a or HsfA8 function as ROS or redox sensors in plants.

ACKNOWLEDGEMENTS

Work at the laboratory of R.M. is supported by funding from The National Science Foundation (NSF- 0431327; NSF-0420033) and The Nevada Agricultural Experimental Station (Publication number 03055517).

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