Mini-review

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# The Hsf world: classification and properties of plant heat stress transcription factors

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**Abstract** Based on the partial or complete sequences of 14 plant heat stress transcription factors (Hsfs) from tomato, soybean, *Arabidopsis* and maize we propose a general nomenclature with two basic classes, i.e. classes A and B each containing two or more types of Hsfs (HsfA1, HsfA2 etc.). Despite some plant-specific peculiarities, essential functional domains and modules of these proteins are conserved among plants, yeast, *Drosophila* and vertebrates. A revised terminology of these parts follows recommendations agreed upon among the authors and representatives from other laboratories working in this field (see legend to Fig. 1). Similar to the situation with the small heat shock proteins (sHsps), the complexity of the *hsf* gene family in plants appears to be higher than in other eukaryotic organisms.

## INTRODUCTION

Heat stress transcription factors (Hsfs) are the terminal components of a signal transduction chain mediating the activation of genes responsive to heat and a large number of chemical stressors (Ritossa 1964; Nover 1991; Morimoto et al 1992). Stress-induced gene expression leads to the rapid accumulation of heat shock proteins (Hsps) which belong to 11 conserved multiprotein families. As molecular chaperones they play a central role not only in protection against stress damage but also in folding, intracellular distribution and degradation of proteins as well as for the function of signal transduction chains (Nover et al 1990, Nover 1991, 1994; Gething and Sambrook 1992; Craig et al 1993; Hartl and Martin 1995; Kimura et al 1995; Waters et al 1996). Though not understood in sufficient detail, the multiplicity of Hsp isoforms within cells, the variability of their expression patterns in different tissues and the complexity of regulatory elements identified in the promoter regions of the corresponding genes are thought to reflect the pleiotropic and indispensible functions of these proteins under stress and non-stress conditions.

The initial concept of a single, constitutively expressed regulatory protein (Hsf) required for *hs* gene activation in yeast (Sorger and Pelham 1988; Wiederrecht et al 1988; Jakobsen and Pelham 1991) and *Drosophila* (Clos et al 1990) had to be revised because of several important observations:

1. Hsfs are coded by small gene families with up to five members in plants (Scharf et al 1990, 1993; Hübel and Schöffl 1994; Czarnecka-Verner et al 1995; Gagliardi et al 1995) and 3–4 members in vertebrates (Rabindran et al 1991; Sarge et al 1991; Schuetz et al 1991; Nakai and Morimoto 1993; see summary by Scharf et al 1994).
2. At least in plants, some of the Hsfs are themselves hs-inducible proteins. This indicates an additional aspect of stress gene activation, possibly by sequential exchange of Hsfs at hs promoters during the stress response.
3. In addition to the control of *hs* gene transcription during the stress response, Hsfs may have other

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**Table 1** Properties of plant HSFs

| HSF class[a] | Ref.[b] | Size | | | | Expression | Structural characteristics[d] | | NLS[f] | HR-C | Access. nos. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mRNA (kb) | Protein[c] (aa) | MW (kDa) | Mr (kDa) | | Linker 1 DBD:HR-A (aa) | Linker 2[e] HR-A:HR-B (aa) | | | |
| HsfA1 | | | | | | | | | | | |
| 1. Lp-Hsf8 | 1-3 | 2.0 | 527 | 57.5 | 68 | constitutive | 32 | 21 | Prox. | + | X67600 |
| 2. At-Hsf1 | 4 | 2.1 | 491 | 54 | 80 | constitutive, slightly hs-induced | 16 + 14 | 21 | (Prox.) | n.d. | X76167 |
| 3. Zm-Hsfa | 5 | 2.3 | truncated | | | constitutive | n.d. | n.d. | n.d. | n.d. | — |
| HsfA2 | | | | | | | | | | | |
| 4. Lp-Hsf30 | 1-3 | 1.4 | 351 | 40.2 | 55 | hs-induced | 14 | 21 | Prox. | + | X67601 |
| 5. Gm-Hsf21 | 6 | n.d. | truncated | n.d. | n.d. | hs-induced | 15 | n.d. | n.d. | n.d. | Z46952 |
| 6. Zm-Hsfb | 5 | 1.6 | 308 | 35.3 | | hs-induced | 15 | 21 | (Prox.) | + | X82943 |
| 7. Zm-Hsfc | 5 | 1.5 | >364 | n.d. | n.d. | hs-induced | 24 | 21 | (Prox.) | ? | - |
| 8. At- Hsf21 | 7 | n.d. | >400 | 46.3 | n.d. | n.d. | 17 | 21 | (Prox.) | n.d. | U68561 |
| HsfB1 | | | | | | | | | | | |
| 9. Lp-Hsf24 | 1-3 | 1.5 | 301 | 33.3 | 45 | hs-induced | 48 | | (Distal) | - | X55347 |
| 10. Gm-Hsf34 | 6 | n.d. | 282 | 31.2 | 37.4 | hs-induced | 40+7 | | (Distal) | - | Z46953 |
| 11. At-Hsf4 | 7 | n.d. | 284 | 31.4 | n.d. | n.d. | 36+7 | | (Distal) | - | U68017 |
| HsfB2 | | | | | | | | | | | |
| 12. Gm-Hsf29 | 6 | n.d. | truncated | n.d. | n.d. | hs-induced | 68 | | n.d. | - | Z46951 |
| 13. Gm-Hsf5 | 6 | n.d. | 370 | 42.1 | n.d. | constitutive | 64+71 | | (Distal) | - | Z46956 |
| 14. Gm-Hsf31 | 6 | n.d. | truncated | n.d. | n.d. | constitutive | 62+79 | | (Distal) | - | Z46955 |

a) Plant species are abbreviated as follows: Lp, *Lycopersicon peruvianum* (wild tomato); At, *Arabidopsis thaliana;* Zm, *Zea mays* (maize), Gm, *Glycine max* (soybean).

b) References: 1. Scharf et al. (1990); 2. Scharf et al. (1993); 3. Treuter et al. (1993); 4. Hübel & Schöffl (1994); 5. Gagliardi et al. (1995); 6. Czarnecka-Verner et al. (1995); 7. Barros et al., unpublished.

c) Mass of the protein is given as number of amino acid residues (aa), the sequence-derived mol. weight (MW) and the apparent mass in SDS-PAGE (Mr.)

d) Abbreviations: DBD, DNA binding domain; HR, region with hydrophobic heptad repeats; NLS putative nuclear localization signal. For details of the linker sequence L1 and L2 see Figs. 1 and 4.

e) For explanation of linker L2 see Fig. 4.

f) Functional tests for NLS are available only for the three tomato Hsfs. Identification of NLS motifs in the other Hsfs is based on sequence comparison only .

functions. This was noticed very early, when *hsf* disruption strains of yeast were found to be inviable also under non-stress conditions (Sorger and Pelham 1988; Wiederrecht et al 1988; Jakobsen and Pelham 1991). In mammals, the predominant Hsf involved in stress gene activation is Hsf1. In chicken, Hsf3 seems to be similar in function, but its expression is limited to distinct tissues (Nakai et al 1995), whereas Hsf2 is involved into developmental expression of chaperone-coding genes during spermatogenesis, hematopoietic differentiation and in early embryonic stages (Sistonen et al 1992, 1994; Sarge et al 1994). Interestingly, even in yeast there are three additional members of the *hsf* family as based on homology of the DNA-binding domain (DBD) (Fig. 3). These are the flocculent suppressor protein Sfl1 (Fujita et al 1989), a putative two-component response regulator Skn7 (Brown et al 1993) evidently involved in the oxidative stress response (Krems et al 1995) and the Mga1 protein found with accession number S47924 in the GenBank. Despite a remarkable homology of their putative DNA-binding domains with that of Hsf1, they cannot replace it functionally.

Following the initial characterization of three tomato Hsfs (Scharf et al 1990, 1993; Treuter et al 1993)) the number of *hsf* clones characterized from plants has rapidly increased (Hübel and Schöffl 1994; Czarnecka-Verner et al 1995; Gagliardi et al 1995; see Table). Based on structural homology and on the evolutionary lineage, we propose a unified nomenclature for the plant Hsfs, which also includes a revision of operative terms used in reference to the functional modules/domains of these proteins. Because many of them are conserved between all eukaryotic Hsfs, this revision follows a general agreement among the authors and representatives of other major laboratories working in the field (see Acknowlegdements).

## GENERAL STRUCTURE OF HSFS AND PLANT-SPECIFIC DIFFERENCES

To our knowledge, Hsfs have been investigated in four plant species , i.e. *Lycopersicon peruvianum* (tomato), *Arabidopsis thaliana*, *Zea mays* (maize) and *Glycine max* (soybean). Comparison of amino acid sequences and functional modules allows the definition of two major classes of Hsfs, each

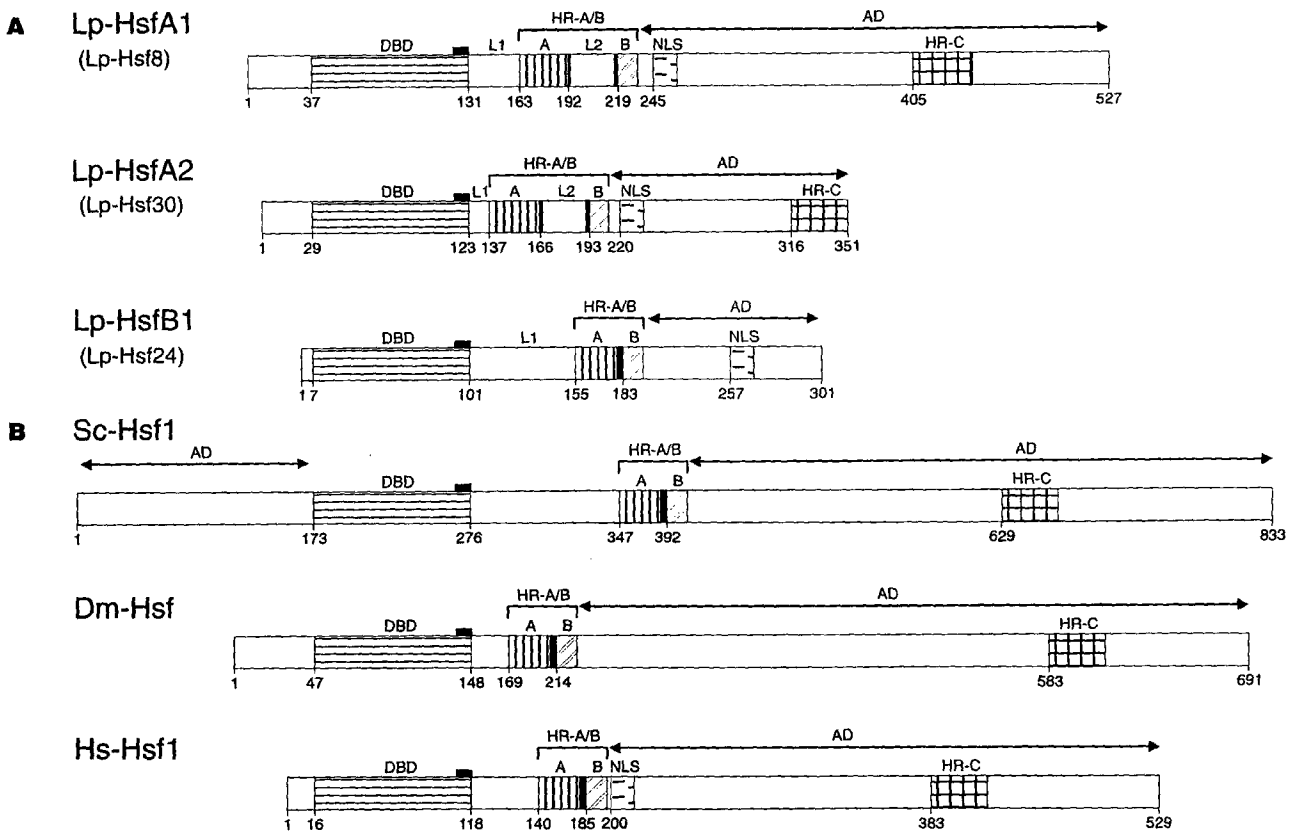
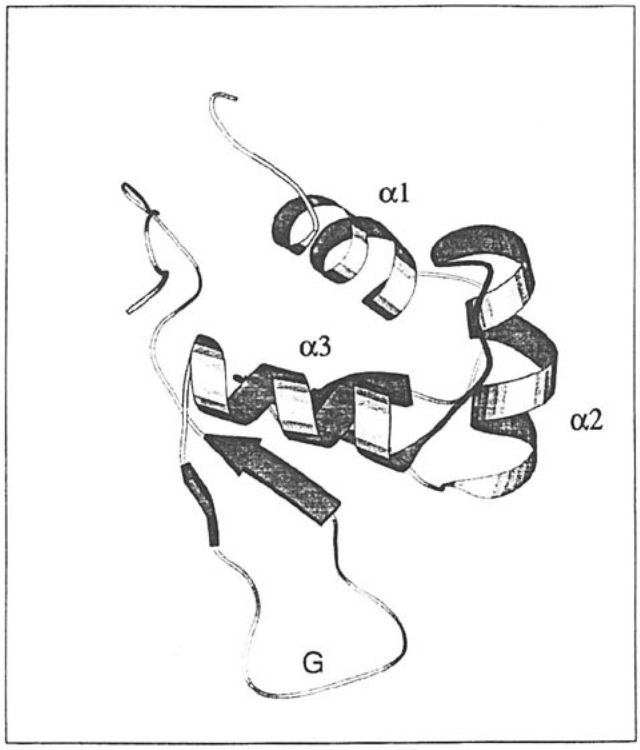
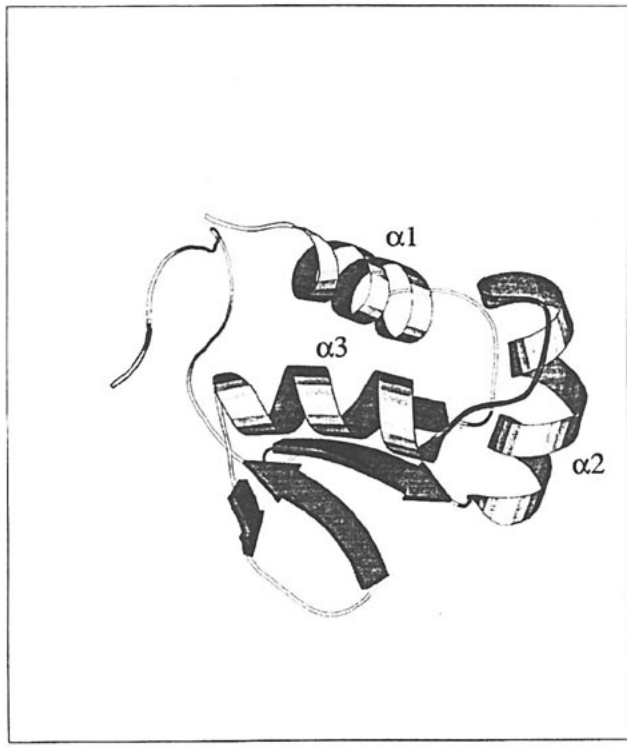
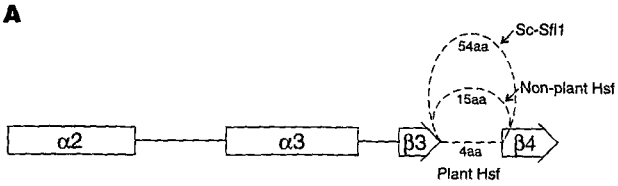



**Fig. 1** Basic structure of three types of plant Hsfs. The block diagram above (A) with common functional modules is based on the structure of the three tomato Hsfs: the new names are used (see Table), but the former names are given in brackets. For comparison, part B represents three examples of non-plant Hsfs, i.e. those from baker's yeast (Sc), *Drosophila* (Dm) and human (Hs). DBD, DNA binding domain; HR-A/B, HR-C, regions with hydrophobic heptad repeats; NLS, nuclear localization signal; AD, activation domain; L1, L2, linker sequences explained in the text. The black bar at the C-terminus of the DBD marks the position of the K/R1 motif mentioned in the text.




**A**




| | | |
|---|---|---|
| **Plant Hsfs** | | T/L |
| 1. | Lp-HsfA1 | ...RQLNTY-GF-RKV---4--WEFA... |
| 2. | Lp-HsfA2 | ...RQLNTY-GF-RKV---4--WEFA... |
| 3. | At-HsfA1 | ...RQLNTY-GF-RKV---4--WEFA... |
| 4. | Lp-HsfB1 | ...RQLNTY-GF-RKI---4--WEFA... |
| **Non-plant Hsfs** | | |
| 5. | Dm-Hsf | ...RQLNMY-GF-HKI--14--IEFSH.. |
| 6. | Hs-Hsf1 | ...RQLNMY-GF-RKV--15--TEFQK.. |
| 7. | Sp-Hsf | ...RQLNMY-GF-HKV--16--LEFA... |
| 8. | Sc-Hsf1 | ...RQLNMY-GF-HKV--15--WQFE... |
| **Yeast Hsf homologous proteins** | | |
| 9. | Sc-Skn7 | ...RQLNKY-DF-HKV--16--WEFQ... |
| 10. | Sc-Mga1 | ...RQLHMY-GF-HKL--20--WKFT... |
| 11. | Sc-Sfl1 | ...RQLNIY-GF-HKV--54--WEFK... |

**B**

**Fig. 2** Structure of the DNA-binding domain. (A) Molscript pictures of the solution structure of the DBD of the yeast Hsf1 (left) and the tomato HsfB1 (right) are shown. They are almost identical except for a flexible loop with an invariant glycine residue between β3 and β4 strands found in the yeast and all non-plant Hsfs (left). (B) Block diagram of the HTH and β3/β4 motifs with position of the introns (arrow) at the end of a3 and the size of the turn/loop (T/L) structure between β3 and β4. In addition to the only functional Hsf1 in yeast, there are at least three other proteins with a potential DBD with high homology to Hsfs (nos 9–11). Two of them have extended loops of 20 (Mga1) and 54 residues (Sfl1), respectively.

Whenever analyzed, an intron was found to separate the coding parts for the HTH motif at or in the invariant Trp codon from the upstream coding sequences for β3. In plants and *Schizosaccharomyces* (nos 1–4 and 7) this is the only intron identified so far, whereas additional introns are found in the hsf genes of *Drosophila* and man (C. Wu, personal communication). For references to the sequence data see Introduction and legend to Figure 3.

Pictures for (A) were kindly provided by J. Schultheiß and O. Kunert (Frankfurt).

further divided into two subclasses. These are class A with Hsfs A1 and A2 and class B with Hsfs B1 and B2. Class A and HsfB1 are common to the four species investigated but probably also to other plants (Table). However, the HsfB2-type represents a tentative assignment of three soybean *hsf* clones, which clearly belong to group B but have no counterpart in the other plant species investigated so far. In case of multiplicity within one subclass, small-case letters may be added for identification, e.g. HsfB2a, b and c for Gm-Hsf29, 5 and 31 respectively (Table).

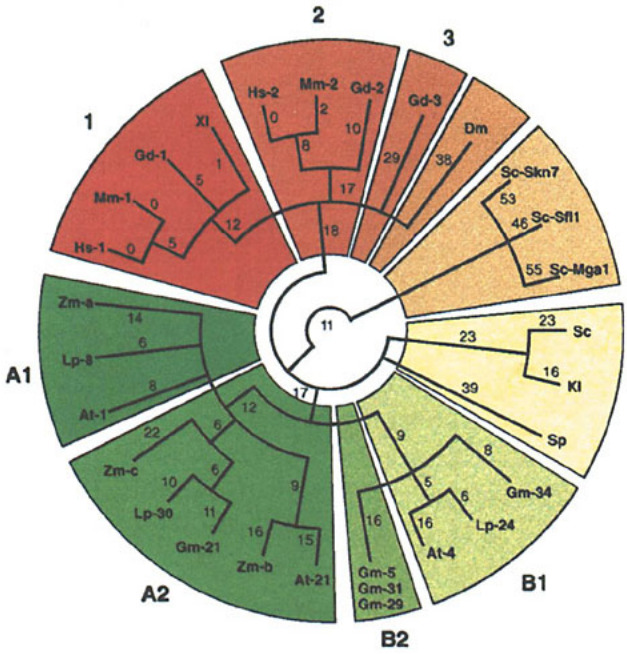



**Fig. 3** Relationship of Hsfs based on amino acid sequence comparison of the DNA binding domains. The consensus tree for all Hsfs was conducted with midpoint rooting using the Phylogenic Analysis Using Parsimony (PAUP) software (Smithonian Institution 1993) with 500 repetitions according to the bootstrap method at the 50% confidence level. Plant Hsf classes A1, A2, B1 and B2 as well as animal Hsf classes 1, 2 and 3 are marked. The figure is based on sequences excluding the variable turn/loop structure between β3 and β4 (Fig. 2). Organisms are abbreviated by a two-letter code based on their scientific names. *Plants*: At, *Arabidopsis thaliana*; Gm, *Glycine max* (soybean); Lp, *Lycopersicon peruvianum* (wild tomato); Zm, *Zea mays* (maize); *animals*: Dm, *Drosophila melanogaster* (fruit fly); Gd, *Gallus domesticus* (chicken); Hs, *Homo sapiens* (human); Mm, *Mus musculus* (mouse); Xl, *Xenopus laevis* (frog); *yeasts*: Kl, *Kluyveromyces lactis*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*. References to sequence information are given in the Table and the Introduction except for Sp-Hsf (Gallo et al 1994), Sc-Mga1 (GenBank acc. no. S47924) and Xl-Hsf (GenBank acc. no. L36924).

The main criteria for the classification are:

1. the assignment to class A and B based on the parsimony analysis of the DNA-binding domain (Fig. 3)
2. the mode of expression (constitutive vs. hs-induced)
3. the length and structure of linker regions L1 and L2 (see Fig. 1 and Table)
4. the fine structure of the oligomerization domain (HR-A/B, Fig. 4)
5. the position of a cluster of basic amino acid residues (K/R), which in the three tomato Hsfs was shown to be essential for nuclear import (NLS).

A number of structural elements are common to all Hsfs (Fig. 1). They are briefly described in the following with special emphasis on peculiarities found in the plant proteins (for reviews see Scharf et al 1994; Wu 1995).

### The DNA-binding domain

Consistent with the conserved promoter recognition sites (HSE) for Hsp coding genes (Nover 1987, 1991), all eukaryotic Hsfs are characterized by an N-terminal DBD formed by an antiparallel four-stranded β-sheet and a three-helical bundle (Fig. 2A). The central, most conserved portion is a helix–turn–helix motif (HTH) essential for DNA recognition (Damberger et al 1994; Harrison et al 1994; Vuister et al 1994a, 1994b; Schultheiss et al 1996). All genomic clones investigated so far contain an intron of variable size inserted immediately after the C-terminus of the HTH motif (Fig. 2B).

A remarkable peculiarity of all plant Hsfs is a deletion of 11 amino acid residues forming an unstructured loop between β-strands 3 and 4 in all non-plant Hsfs. Thus, in plants the β3- and β4-strands are connected by a turn of only four amino acid residues instead of a loop of variable size in all other proteins of this family (Fig. 2A, B).

The key argument for the organization of the plant Hsfs into two classes stems from the parsimony analysis of amino acid residues of the DBD. The assumption that underlies this method is that the primary amino acid sequences, e.g. of the Hsf DBDs, contain information regarding specific aspects of protein structure and functional specialization. Interpretation of this type of analysis must take into account the fact that the derived groupings or proteins reflect both the uniqueness of functional classes and the evolutionary distances between various organisms. One measure of the usefulness of this method is that the expected groupings are observed for Hsf types 1 and 2 of vertebrates where functional classes are represented by a single gene. This result also indicates that information regarding functional classes can be obtained from the analysis of the DBD alone.

Phylogenic trees of Hsfs constructed either by the neighbor joining approach of the CLUSTAL W program (Thompson et al 1994, data not shown) or the parsimony approach of the PAUP program consistently indicated that all cloned plant Hsfs can be assigned to one of two groups, A or B (Fig. 3), which are distinct from any of the previously characterized proteins of non-plant origin. No major differences were seen when the analysis included the variable loops between β-strands 3 and 4 or not.

### The hydrophobic heptad repeat region

A linker region of variable length and sequence (L1, Flick et al 1994) connects the DBD with a region of hydrophobic heptad repeats (HR-A/B) evidently responsible for the oligomerization of Hsfs (Sorger and Nelson 1989; Peteranderl and Nelson 1992; Zuo et al 1994; K.D. Scharf, unpublished data). This function and the particular heptad repeat pattern of large hydrophobic amino acid residues led to speculations about two adjacent peptide motifs (A and B) with potential for coiled-coil interactions. Both are connected by a short flexible linker (Sorger and Nelson 1989; Rabindran et al 1993; Zuo et al 1994, 1995). In support of the functional separation of the two parts, the plant class A Hsfs are discriminated from all others by an insertion of 21 amino acid residues in this linker, which gives rise to a second heptad repeat pattern (stars and open circles in Fig. 4). In contrast to this, class B Hsfs and all non-plant Hsfs characterized so far lack this insertion and have a single, continuous heptad repeat pattern (closed circles in Fig. 4), such that the entire region could form a single coiled-coil structure, although there is no direct evidence to support this (R.H. Peteranderl and H.C.M. Nelson, personal commununication). In view of the important function of this region not only for oligomerization but evidently also for the activity control (Jakobsen and Pelham 1991; Chen et al 1993; Zuo et al 1994), it will be interesting to characterize the structure of the more extended version of the HR-A/B region of class A plant Hsfs and to elaborate the role of the highly conserved charged residues in Figure 4.

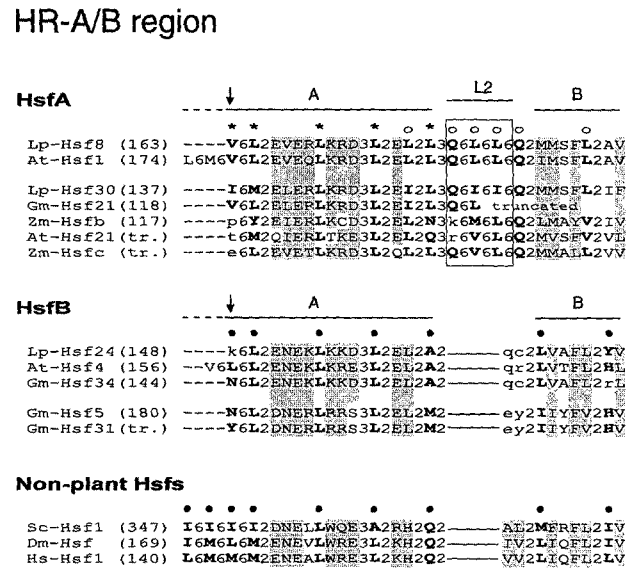



**Fig. 4** Sequence comparison of the HR-A/B regions. The amino acid residue in the HR-A/B region used to define the length of L1 is indicated by an arrow. As indicated in some cases, the heptad repeat pattern may extend beyond this position, e.g. in the At-Hsf1 or the non-plant Hsfs. The heptad repeat positions are marked. Formally, two overlapping repeat patterns are found in class A Hsfs (stars and open circles respectively), whereas all others have a single continuous pattern (closed circles). Highly conserved residues which are not in the frame of the repeats are marked by shading. The characteristic 21 amino acid residue insert of the class A Hsfs is boxed. tr, some sequences are incomplete in their N-terminal or C-terminal parts.

### Potential nuclear localization signals motifs

Practically all Hsfs contain two clusters of basic amino acid residues (K/R motifs) considered as putative nuclear localization signals (NLS, see Fig. 1). K/R1 represents the conserved C-terminal part of the DBD, whereas K/R2 is positioned in the activator domain either adjacent to the HR-A/B region (proximal position) or, in the class B Hsfs, more distal, i.e. close to the C-terminus. The proximal position of the K/R2 motif is also typical for vertebrate Hsfs. Functional tests of the human Hsfs 1 and 2 with mutation or deletion of the potential NLS motifs led to the conclusions that both K/R motifs (Sheldon and Kingston 1993), or only the K/R1–motif is required for nuclear import (Zuo et al 1995). In contrast, our studies with mutants of the tomato Hsfs A1 and A2 identified the K/R2 motif as the only NLS (Lyck et al submitted). In addition, a mutant form of the tomato HsfB1 with a deletion of the distal K/R2 motif is defective in nuclear import. Unfortunately, other plant Hsfs were not investigated in this respect, i.e. identification of the putative NLS motifs is based on sequence comparison only.

### The activation domain

The region C-terminal to the oligomerization domain of the Hsfs contains the NLS and the transcriptional activation domain (AD). The AD is not as well defined as other parts of the Hsf. Frequently, it comprises positive (activator) and negative (repressor) elements (Nieto-Sotelo et al 1990; Nakai and Morimoto 1993; Rabindran et al 1993; Hoj and Jakobsen 1994; Shi et al 1995; Zuo et al 1995; Wisniewski et al 1996; Lyck et al, unpublished data). Though evident similarities are almost lacking, Hsfs were shown to function more or less properly in heterologous systems, e.g. the *Drosophila* Hsf and the tomato Hsfs A1 and A2 in yeast (O. Boscheinen et al, unpublished data), the human Hsf1 and the *Drosophila* Hsf in tobacco protoplasts (Treuter et al 1993), the human Hsf1 in *Drosophila* cells (Clos et al 1993), and the *Arabidopsis* HsfA1 in mammalian and insect cells (Hübel et al 1995). The essential functional elements seem to reside in or close to a third hydrophobic heptad repeat region (HR-C) which is also found in the plant class A Hsfs. Most intriguing are short peptide motifs rich in aromatic, large

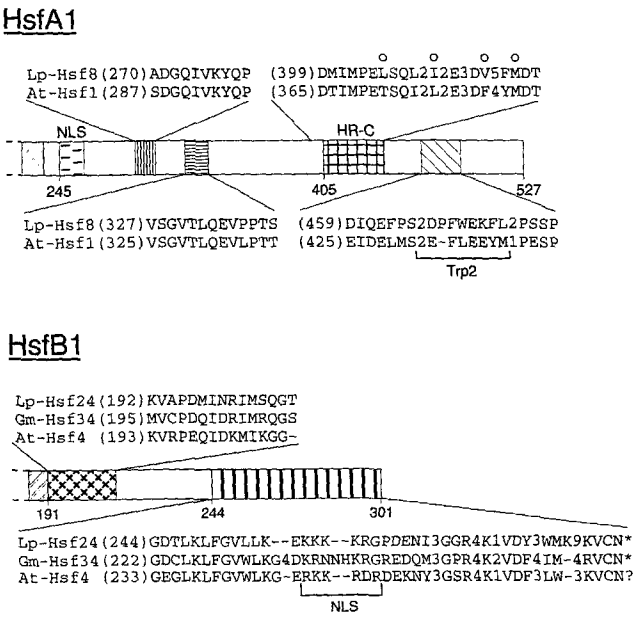



**Fig. 5** Sequence conservation in the C-terminal parts of Hsfs A1 from tomato and *Arabidopsis* (above) as well as of Hsfs B1 from tomato, *Arabidopsis* and soybean (below).
The C-terminal parts are shown as block diagrams based on the two tomato Hsfs. Sequences are given with numbers indicating the starting positions in the given Hsf (for references see Table).

*h*ydrophobic and *a*cidic amino acid residues (Treuter et al 1993). They were shown to be important for the activity, at least of the tomato Hsfs A1 and A2. These **AHA** motifs are also found in the center of activation domains of the human Hsf1 (Newton et al 1996), the yeast Hsf (Chen et al 1993) and an increasing number of other transcription factors, e.g. GAL4, GCN4, VP16, Sp1, RelA, C/EBP, p53 and others (for a summary see Nover and Scharf 1997).

Considering our very limited knowledge about the functional elements of the activation domains in other plant Hsfs, it is worth noticing that there are marked regions of sequence homology between tomato and *Arabidopsis* Hsfs A1 on the one hand and among class B1 Hsfs from *Arabidopsis*, soybean and tomato on the other hand (Fig. 5). The close relatedness of the latter three is also evident from Figure 3 and from sequence conservation in the L1 region. The functional significance of these conserved regions remains to be investigated.

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