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Interaction between the *Arabidopsis thaliana* heat shock transcription factor HSF1 and the TATA binding protein TBP

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Abstract The heat shock factor (HSF1) is the central regulator of the heat stress (hs) response and is required for stimulating the transcription of the hs genes and consequently the expression of heat shock proteins. To promote the polymerase II-dependent transcription of the hs genes, HSF has to communicate with the basal transcription machinery. Here, we report that the *Arabidopsis thaliana* HSF1 interacts directly with TBP, the general TATA box binding transcription factor, as shown by affinity chromatography and electrophoretic mobility shift analyses in vitro. An in vivo interaction between *At*HSF1 and *At*TBP1 was suggested by results employing the yeast two-hybrid system.

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Key words: Heat shock; Heat shock factor 1; Transcription factor; TATA box binding protein; *Arabidopsis thaliana*

1. Introduction

The transcriptional activation of genes encoding heat shock proteins (HSPs) is a highly conserved response to heat and other environmental stresses in all organisms [1–4]. HSPs function as molecular chaperones in various cellular processes [5,6]. The expression of the HSPs is mediated by members of the heat shock transcription factor family (HSFs). In plants and other higher eukaryotes, small families of HSF and HSF-like genes have evolved [7–12] suggesting distinct roles for the different HSFs [13–15]. In contrast, only one *Hsf* gene is described for yeasts and *Drosophila*, and the yeast *Hsf* gene was demonstrated to be essential for the survival of yeast at normal temperatures [16–19]. Furthermore, recent data suggest an involvement of *Drosophila* HSF in processes such as oogenesis and early larval development under normal growth conditions [20]. HSF1 is a constitutive protein in several organisms including *Arabidopsis* [10] and is maintained in an inactive monomeric, but stress-responsive conformation in the cytoplasm [1]. After activation, trimeric HSF cooperatively binds to heat shock elements (HSE) located in the proximity of the TATA element in the promoters of hs genes. Molecular models for the activation of HSF suggest an exposure of a C-terminal transactivation domain (CTA), by either intramolecular conformational rearrangements and/or dissociation of a negative regulatory factor [1,18,21–23]. Multiple and functionally distinct activation domains were mapped in HSF1 of human and yeast HSF [24,25].

A rapid transactivation of TATA box containing hs promoters [26,27], with for example each uninduced *Drosophila hsp70* promoter containing TBP [28] and one paused RNA polymerase II [29], might require an interaction between HSF trimers and factors of the basal transcription machinery. A target candidate is the general transcription factor TBP, which is the first promoter binding component serving to position and assemble the complete transcription complex [30]. Recently, TBP was indeed found to interact with HSF at the *Drosophila hsp70* promoter [31]. The binding of TBP to the TATA box is the major rate-limiting step in the transcription initiation process [32,33]. The efficiency of TBP-TATA binding could therefore be increased by transactivating factors such as HSF. Several other transcriptional activator proteins including VP16, adenovirus large E1A protein, the HIV-1 transactivator protein Tat, and p53 have been previously shown to interact directly with TBP [34–37]. TBP is a component of the multisubunit complex TFIID, also containing several TBP-associated factors (TAFs) [30].

In the present study we examined whether a plant HSF, HSF1 from *Arabidopsis thaliana* (*At*HSF1) [10], is able to interact with the two known constitutively expressed *Arabidopsis* TATA binding proteins, TBP1 and TBP2 (*At*TBP1, *At*TBP2) [38]. Both affinity chromatography and DNA binding assays performed with recombinantly expressed proteins in *Escherichia coli* show an *At*HSF1-*At*TBP1 and *At*HSF1-*At*TBP2 interaction in vitro. In addition, the use of the yeast two-hybrid system provides evidence that HSF1 and TBP interact also in vivo.

2. Materials and methods

2.1. Cloning and expression of recombinant AtHSF1 protein

*At*HSF1 cDNA was expressed as His-tagged protein (Qiaexpressionist, Qiagen) [10] in *E. coli* and purified to homogeneity by repeated Ni^{2+} affinity chromatography. Recombinant 6×His*At*HSF1 protein was dialyzed in a buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 10% glycerol, to eliminate contaminating Ni^{2+} ions released from the affinity column.

2.2. Cloning and expression of recombinant AtTBP1 and AtTBP2 proteins

*At*TBP1 and *At*TBP2 cDNAs provided by N.-H. Chua were expressed in *E. coli* using the pET system (Novagen), purified by binding to Ni^{2+} Sepharose, and dialyzed against binding buffer containing 20 mM Tris-HCl pH 7.8, 100 mM NaCl, 5 mM β-glycerophosphate, 5 mM $MgCl_2$, 1 mM NaF, 10% glycerol, 0.01% NP40, 5 μg/ml leupeptin, aprotinin, pepstatin, and chymostatin.

2.3. In vitro binding assays

Recombinant *At*HSF1 protein was coupled to CnBr-activated Sepharose 4B (Pharmacia) at a concentration of 4 mg/ml. 25 μg Sepharose-coupled *At*HSF1 protein was incubated in binding buffer for 1 h at 4°C with 25 μg of recombinant *At*TBP1 or *At*TBP2 protein. As controls, recombinant glucuronidase protein (GUS; prepared as de-

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scribed in [39]) and BSA (Sigma) were utilized. The *At*HSF1-Sepharose was washed three times each with 300 μl binding buffer containing 200 mM NaCl. Proteins bound to the *At*HSF1 matrix were eluted with binding buffer containing either 0.5 or 1.0 M NaCl. Volumetrically identical aliquots of eluted samples were electrophoresed on 10% SDS-PAGE and proteins were visualized by Coomassie staining.

2.4. Electrophoretic mobility shift assay (EMSA)

Oligonucleotide probes containing either consensus HSEs (5′-GGATCCTAGAAGCTTCCAGAAGCTTCTAGAAGCAGATC-3′) or mutated HSE sequences (HSEmut; 5′-GGATCCTATAAGCTTACATAAGCTTATATAAGCAGATC-3′) were processed as described [10]. *At*TBP1 and *At*TBP2 binding to HSE probes was tested by incubation of 12 000 cpm of radiolabelled DNA with either 1 μg *At*TBP1 or *At*TBP2 protein in retardation buffer (20 mM Tris-HCl pH 7.8, 100 mM NaCl, 2 mM EGTA, 5 mM β-glycerophosphate, 2 mM $MgCl_2$, 0.5 mM DTT, 1 mM NaF, 0.1 mM Na_3VO_4, and 10% glycerol) containing 0.75 μg poly(dIdC) unlabelled non-specific competitor DNA (Sigma) in a total volume of 20 μl. The reactions were incubated on ice for 1 h followed by 14 min at room temperature.

To detect protein-protein interactions between *At*HSF1 and *At*TBP1 or *At*TBP2, EMSA reactions containing 1 μg of each protein were used. Protein binding was allowed to proceed at room temperature for 10 min in binding buffer. To the reactions, retardation buffer containing radiolabelled oligonucleotide probes (12 000 cpm of either HSE or HSEmut) and 0.75 μg poly(dIdC) was added to a final volume of 20 μl. Following incubation at room temperature for 10 min, the reactions were loaded on native 4% polyacrylamide gels, electrophoresed in 0.5×TBE at 250 V for 2.5 h, vacuum dried and subjected to autoradiography.

2.5. Yeast two-hybrid system

Standard manipulations of yeast were performed as described in *Methods in Yeast Genetics* [40]. Full-length *At*HSF1 cDNA and a C-terminal *At*HSF1 deletion encoding 388 amino acids (*At*HSF1–E388) were cloned as *Sal*I fragments into pEG202 (HIS^+) expressing *At*HSF1 and *At*HSF1–E388 proteins with a LexA DNA binding domain fusion. *Bam*HI-*Sal*I fragments of the *Drosophila* Bicoid cDNA (provided by R. Finley and R. Brent) were cloned into pEG202. *At*TBP1 cDNA was cloned as a *Eco*RI-*Xho*I fragment into pJG4-5 (TRP^+) under the control of a galactose-inducible promoter expressing *At*TBP1 fusion proteins carrying an activation domain [41]. All constructs were transformed into yeast strain EGY48 (*MAT*a *trp1 ura3 his3 LEU2::pLexAop6-LEU2*) containing the reporter plasmid pSH18-34 (URA^+), which directs expression of *lacZ* via *GAL1* promoter-localized *LexA* operators. Transformants were selected for on medium containing galactose, but lacking histidine, uracil, tryptophan, and leucine. All yeast plates were incubated for 2 days at 30°C. An interaction between *At*HSF1 and *At*TBP1 activates transcription of *leu2* leading to selectable leucine prototrophy. Reporter β-galactosidase activity was detected using CPRG (chlorophenol red-β-D-galactopyranoside, Boehringer Mannheim) as substrate in three independent measurements as described [42].

3. Results

3.1. AtTBP1 and AtTBP2 specifically bind to AtHSF1 in vitro

Affinity chromatography was employed to detect a possible direct in vitro interaction of plant HSF1 (*At*HSF1) with the TATA box binding proteins *At*TBP1 and *At*TBP2 of *Arabidopsis thaliana*. A mock matrix was used to examine non-specific binding of *At*HSF1, *At*TBP1, *At*TBP2, BSA and recombinant GUS, the latter two serving as negative controls (Fig. 1A, lanes 4–8). Except for BSA (lane 8), none of the proteins utilized was able to bind to this matrix. Recombinant *At*HSF1 protein coupled to CnBr-activated Sepharose was then used as an affinity matrix from which specifically bound proteins were eluted by an increasing salt gradient. Both *At*TBP isoforms were able to bind to *At*HSF1 (Fig. 1B, lanes 2,3,5,6). Interestingly, *At*TBP1 and *At*TBP2 appear to have

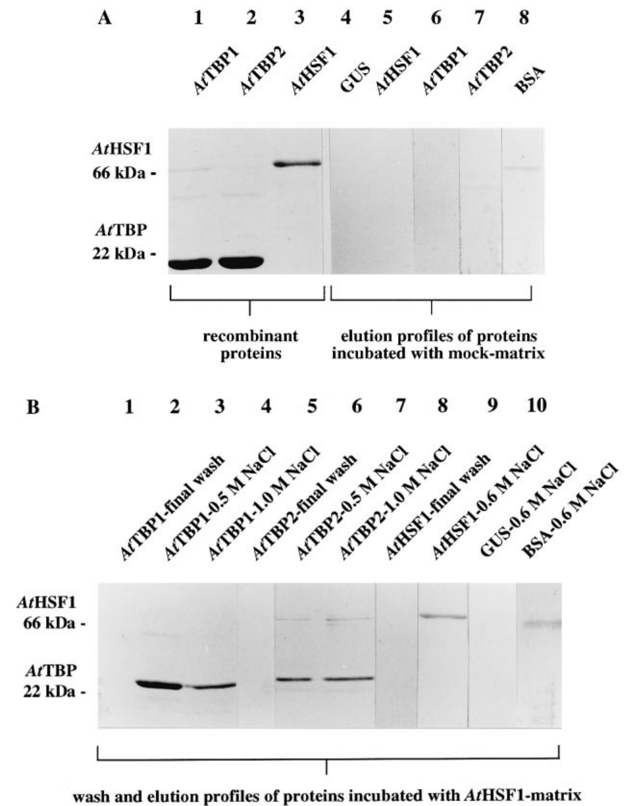

Fig. 1. In vitro binding assays. A: Empty CnBr-activated Sepharose was used as a mock matrix to test for possible unspecific binding capacities of the indicated proteins. Recombinant *At*HSF1, *At*TBP1, and *At*TBP2 proteins are shown as standards. *At*TBP1 and *At*TBP2 were incubated with the mock matrix and eluted by a salt gradient. *At*HSF1, GUS, and BSA were treated identically and served as controls. B: Recombinant *At*HSF1 was coupled to CnBr-activated Sepharose and incubated with recombinant *At*TBP1 or *At*TBP2. *At*HSF1, GUS, and BSA were used as controls to test for the specificity of the binding reaction. After incubation with the *At*HSF1 matrix, bound proteins were eluted by two salt concentrations. The absence of residual protein was verified by inspection of the final washing step.

different affinities for *At*HSF1 (compare lane 2 with lane 5). 25% of *At*TBP1, but only 10% of *At*TBP2 total protein used in the binding assay bound to *At*HSF1. Due to a lack of knowledge about structural differences and cellular functions of *At*TBP1 and *At*TBP2 the differences in their affinities for *At*TBP1 for *At*HSF1 cannot be explained at present.

The complete lack of unspecifically bound *At*TBP1, *At*TBP2, and *At*HSF1 proteins to the *At*HSF1 matrix prior to elution was verified by inspection of the final washing steps (lanes 1,4,7). *At*HSF1 specifically bound to the *At*HSF1 matrix which was completely eluted using 0.6 M NaCl (lane 8). Considering initial protein amounts in the reaction, the binding efficiency was determined to be only 7%. Again, BSA showed a binding competence to the *At*HSF1-Sepharose (lane 10), which was probably due to the high glycerol content of the BSA buffer.

3.2. AtHSF1:HSE complexes migrate differently in the presence of AtTBP in EMSA analyses

In EMSA analyses, we exploited the capacity of *At*HSF1 to bind to consensus HSE and not to mutated HSE, leading to specific *At*HSF1:HSE complexes (Fig. 2A, lanes 1,2). How-

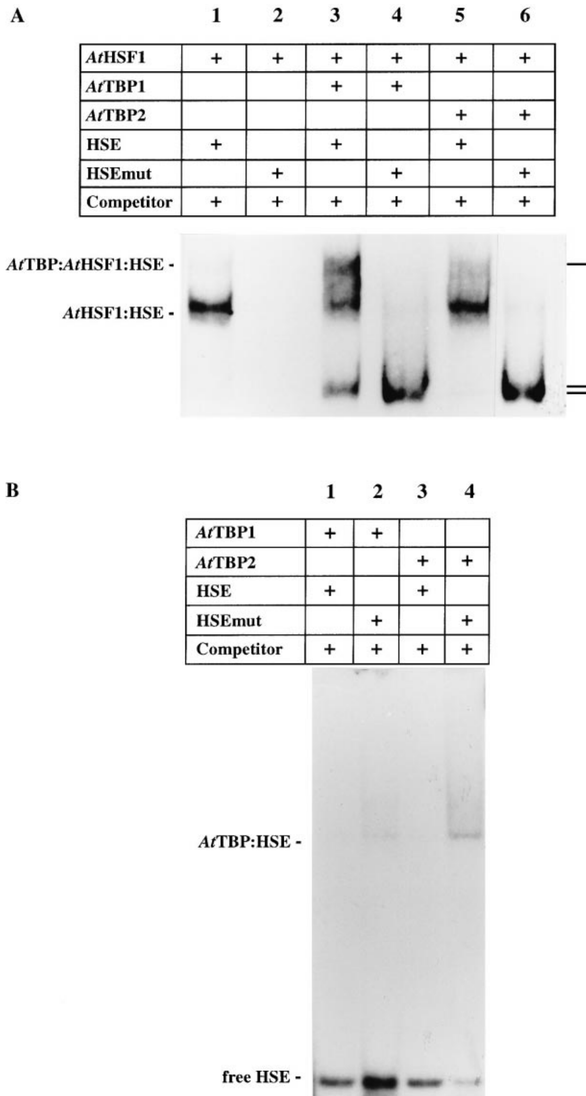

Fig. 2. Electrophoretic mobility shift analysis (EMSA). All reactions were separated on native polyacrylamide gels and the bands were visualized by autoradiography. A: Recombinant *At*HSF1 and *At*TBP1 or *At*TBP2 proteins were incubated with radiolabelled HSE oligonucleotides carrying either consensus HSE (HSE) or mutated (HSEmut) sequences. The single bar depicts *At*TBP:*At*HSF1:HSE complexes, the double bar depicts a faster migrating protein:DNA complex. B: Recombinant *At*TBP protein was examined for intrinsic DNA binding activity. Therefore, either *At*TBP1 or *At*TBP2 protein was incubated with radiolabelled HSE or HSEmut.

ever, in the presence of equal stoichiometric amounts of *At*TBP1 or *At*TBP2 protein, these complexes are further retarded (lanes 3,5; marked by a single bar). To increase the separation of the formed protein:DNA complexes the remaining free DNA probes were allowed to exit the gel. In agreement with the data obtained in the binding studies described above, the formation of HSE:*At*HSF1:*At*TBP complexes was less prominent in the presence of *At*TBP2 (Fig. 2A; compare lane 3 with lane 5). The generation of these new complexes was not dependent on the order in which the components of the binding reaction were mixed and incubated. In the binding reactions loaded in lanes 3–6 (Fig. 2A), preformation of *At*HSF1:HSE complexes was allowed prior to the addition of *At*TBP1 or *At*TBP2 protein. Similar complexes were formed when *At*HSF1 and *At*TBP1 or *At*TBP2 proteins were incubated prior to the addition of radiolabelled HSE (data not shown). The HSE:*At*HSF1:*At*TBP complexes were absent in reactions containing mutated HSE (lanes 4,6). It should be noted that in the binding reactions containing *At*HSF1 and *At*TBP1 or *At*TBP2 proteins (except for the reaction containing *At*HSF1, *At*TBP2, and HSE; lane 5), an additional fast migrating complex appeared (Fig. 2A, lanes 3,4,6; marked by a double bar). This complex can be attributed to the binding of *At*TBP1 and *At*TBP2 to mutated HSE, since identical complexes were observed in control experiments studying *At*TBP1 and *At*TBP2 binding to consensus and mutated HSE in the absence of *At*HSF1 (Fig. 2B). These data confirm the ability of *At*TBP1 and *At*TBP2 to bind to a TATAA-like sequence present in mutated HSE (lanes 2,4). As expected, *At*TBP1 and *At*TBP2 were unable to bind consensus HSE (lanes 1,3). A fast migrating complex in reactions containing *At*HSF1, *At*TBP1, and consensus HSE remains elusive (Fig. 2A, lane 3). It seems possible that due to the observed higher affinity of *At*HSF1 for *At*TBP1 in comparison to *At*TBP2, *At*HSF1 might preferentially recruit *At*TBP1 to bind to consensus HSE. As a result, a partial displacement of *At*HSF1 from the complex could occur. However, it cannot be excluded that a minor fraction of contaminating *E. coli* proteins, which may co-purify with the His-tagged protein from Ni-NTR columns [43], accounts for the extra complex.

3.3. The yeast two-hybrid system detects an AtHSF1-E388 interaction with AtTBP1 in vivo

*At*HSF1 and a C-terminally truncated protein (*At*HSF1–E388) were co-expressed with *At*TBP1 in yeast auxotrophic for the selectable leucine marker. The expression of both

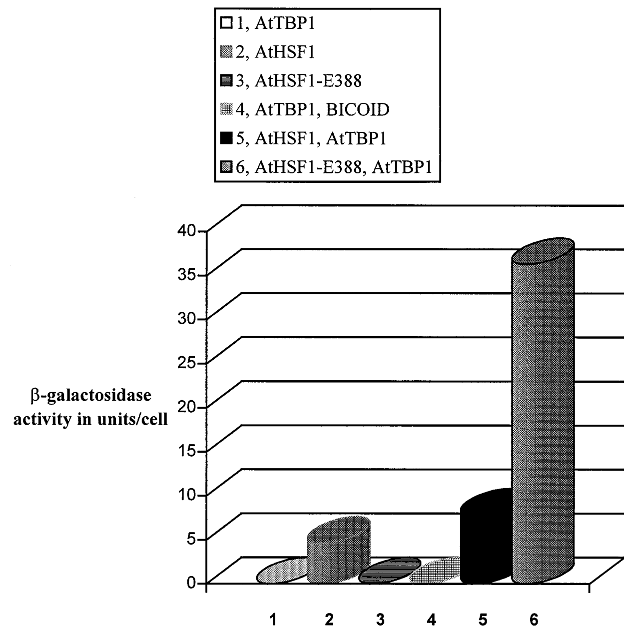

Fig. 3. Activity of β-galactosidase, a second reporter for protein: protein interaction in the yeast two-hybrid system. Enzymatic activity of β-galactosidase in strains expressing *At*HSF1, *At*TBP1, *At*HSF1–E388, *At*TBP1:Bicoid, *At*HSF1:*At*TBP1, and *At*HSF1–E388:*At*TBP1 was quantitated using CPRG as substrate in three independent measurements. Activities were calculated as units β-galactosidase per cell and the values were normalized for a weak background activity of *At*HSF1.

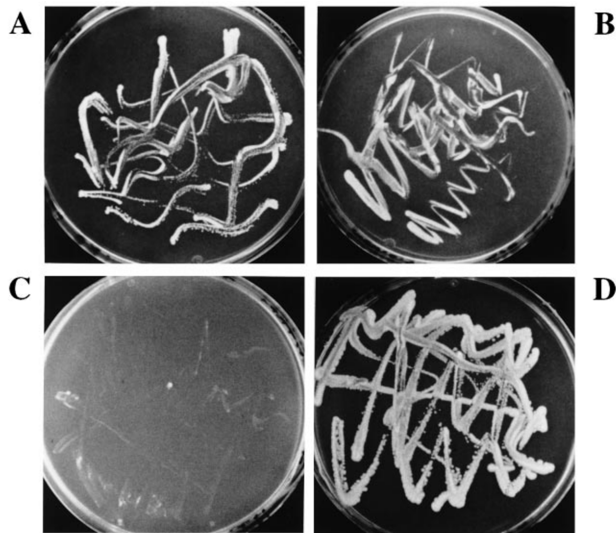

Fig. 4. In vivo interaction of *At*HSF1-*At*TBP1. Using the two-hybrid system, yeast strains co-expressing *At*HSF1 and *At*TBP1 or *At*HSF1–E388 and *At*TBP1 fusion proteins were plated onto medium lacking leucine to test for activation of the leucin reporter gene (A,C) or on medium supplemented with leucine (B,D). Strains co-expressing either the C-terminally truncated *At*HSF1–E388 and *At*TBP1 (A,B) or full-length *At*HSF1 and *At*TBP1 (C,D) were tested. Yeast growth indicates protein:protein interaction between *At*HSF1–E388 and *At*TBP1 (A), resulting in activation of the *leu2* gene promoter and hence leucine prototrophy.

*At*HSF1 fusion proteins in the yeast was confirmed by Western analyses (data not shown). Full-length *At*HSF1 expressing yeast showed a slow growth on medium lacking leucine and also expressed detectable levels of β-galactosidase activity (data not shown; Fig. 3, column 2), indicating an intrinsic potential to autoactivate the reporter genes. However, yeast co-expressing *At*HSF1–E388 and *At*TBP1 were able to grow on medium lacking leucine comparable to growth observed on medium containing leucine (Fig. 4A,B). The photometric inspection of both cultures in liquid medium revealed similar growth rates (stationary culture with $OD_{600} = 2.3$ is obtained after 30 h). In contrast, growth potential in the absence of leucine of yeast containing *At*HSF1 and *At*TBP1 fusion proteins was not significantly higher than growth of yeast containing only *At*HSF1 (Fig. 4C). This growth potential of the *At*HSF1 strain can be attributed to the intrinsic capacity of *At*HSF1 for autoactivation of the selection gene. On medium containing leucine, growth of *At*HSF1 and *At*TBP1 expressing yeast is not affected (Fig. 4D). As a control, *At*TBP1 was co-expressed with a transcriptionally inert fragment of the *Drosophila* Bicoid protein. On medium lacking leucine, no growth was observed (data not shown). To determine the relative strength of the two-hybrid interaction, the activity of expressed β-galactosidase was quantified (Fig. 3). Yeast expressing *At*HSF1–E388 and *At*TBP1 exhibited a 10-fold increase in *lacZ* reporter gene activation. The measured values for yeast containing *At*HSF1 and *At*TBP1 (column 5) and *At*HSF1–E388 and *At*TBP1 (column 6), respectively, were normalized for the weak intrinsic potential of *At*HSF1 to stimulate the *lacZ* promoter (4.6 units β-galactosidase/cell) in the absence of *At*TBP1. *At*TBP1, *At*HSF1, and *At*HSF1–E388 were verified to have no intrinsic potential to activate *lacZ* expression (columns 1–3). Control yeast expressing *At*TBP1 and Bicoid showed no *lacZ* activation (column 4).

4. Discussion

In this work, data obtained in biochemical and genetic analyses suggest a direct interaction between plant HSF1 and TBP in vitro and in vivo. Both *At*TBP isoforms bind to *At*HSF1 protein in affinity assays. In addition, DNA:protein complexes of *At*HSF1 and HSE are further retarded in EMSA in the presence of *At*TBP protein. Recombinant *At*HSF1 protein expressed in *E. coli* is present in a trimeric state after purification in vitro [10]. HSF trimers represent the active form capable of communicating a stress signal to the heat shock genes [1]. Both *At*TBP1 and *At*TBP2 were able to bind to trimeric *At*HSF1 in vitro. This interaction was not dependent on the state of *At*HSF1 with respect to DNA binding. TBP binds either to DNA-bound *At*HSF1 or to free *At*HSF1 in solution. This capacity may indicate that *At*HSF1 is involved either in recruiting *At*TBP to the promoter and/or in stimulating transcription. A capacity for recruiting TBP has also been attributed to other transcriptional activators such as VP16 [30]. Further support for a HSF1-TBP interaction comes from footprinting analyses of the *Saccharomyces cerevisiae Hsp82* promoter, in which a different TATA binding activity of TBP was observed under heat stress. Immediately after a heat stress the efficiency of TATA binding is increased, and this effect is slowly reversed during recovery phase [44].

Alternatively, our data are also compatible with a model suggesting that *At*HSF1 binding is governed by promoter-bound *At*TBP. Chromatin reconstitution experiments using reporter gene constructs under the control of a *Hsp70* promoter suggest that TFIID is able to access nucleosomal DNA and subsequently alleviates binding of HSF to HSEs. This binding is required for the derepression of chromatin and transcriptional activation of the HSE-controlled reporter gene [45,46]. On the other hand, TFIID, a complex containing TBP, is unable to stimulate transcription in the absence of a bound transcriptional activator protein, probably as a result of its low stability binding to the TATA box. The fusion of a heterologous DNA binding domain to TBP resulted in a strong permanent binding to the promoter and increased transcription of a reporter gene. This emphasizes the importance and functional role of a transcriptional activator under natural conditions [32,33]. The affinity of TBP to the TATAA sequence is about 1000-fold lower compared to other sequence-specific DNA binding proteins [47]. An interaction of TBP with sequence-specific activator proteins such as HSF could enhance the assembly of the remaining factors of the transcription pre-initiation complex. This is supported by the finding that TBP is bound to a *Drosophila* hs promoter already in the absence of stress [28,48]. Such an interaction between a basal transcription factor and a co-activator could either directly stabilize TBP-TATA binding or stimulate the dissociation of TBP dimers which were detected in vivo and are unable to bind to DNA [30,49,50]. In each case the functional mechanisms of HSF-TBP interaction would ultimately increase the rate of transcription initiation, which is a prerequisite for the fast expression of hs genes under stressful conditions.

The interaction between HSF and TBP in vivo was indicated in our experiments using the yeast two-hybrid system co-expressing *At*TBP1 and a C-terminally truncated form of *At*HSF1 (*At*HSF1–E388). The difference between *At*HSF1–E388 and full-length *At*HSF1 in their ability to interact with

*At*TBP1 in vivo may be the result of conformational alterations. Only the truncated *At*HSF1–E388 seems to be folded in a conformation accessible for *At*TBP1. This result appears to be in contrast to the biochemical studies showing interaction of TBP and full-length AtHSF1 in vitro. However, one has to consider that recombinant *At*HSF1 used in the in vitro binding experiments might be derepressed in its DNA and TBP binding activities following expression and purification in *E. coli*. The importance of C-terminal regions in the negative regulation of HSF activity has been shown for HSFs of *Drosophila* and chicken. Deletions in this region lead to constitutively DNA binding and transcription-competent HSF [1,9,51] which underscores that the potential to communicate with other factors of the transcription machinery is controlled via C-terminally regulated domains in HSF. In the absence of the hybrid-TBP-protein full-length *At*HSF1 expressed in yeast shows only a weak activity for transcriptional stimulation, suggesting that *At*HSF1 has an intrinsic but repressed capacity to interact with the yeast native TATA box complex. This potential is also repressed in the presence of hybrid *At*TBP1 in yeast. It seems possible that other factors are required in stoichiometric amounts for derepression of the TBP-inaccessible conformation of *At*HSF1 in the yeast two-hybrid system. In addition, not only the conformation but also post-transcriptional modifications are known to contribute to *At*HSF1 regulation. Full-length *At*HSF1 expressed in yeast could therefore be negatively regulated, e.g. through phosphorylation. It was shown for human, mouse, and *Arabidopsis* HSF1 to be phosphorylated in homologous systems under non-stress conditions which might serve to maintain/convert HSF1 in the inactive form [52–54].

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