The target genes of the heat shock transcription factor (HSF) contain a cis-acting sequence, the heat shock element (HSE), which consists of multiple inverted repeats of the sequence 5′-nGAAn-3′. Using data acquired in this and a previous study, we have identified the HSEs in 59 of 62 target genes of *Saccharomyces cerevisiae* Hsf1. The Hsf1 protein recognizes continuous and discontinuous repeats of the nGAAn unit; the nucleotide sequences and configuration of the units diverge slightly among functional HSEs. When *Schizosaccharomyces pombe* HSF was expressed in *S. cerevisiae* cells, heat shock induced *S. pombe* HSF to bind to various HSE types, which properly activated transcription from almost all target genes, suggesting that the *S. pombe* genome also contains divergent HSEs. Human HSF1 induced the heat shock response via HSEs with continuous units in *S. cerevisiae* cells but failed to do so via HSEs with discontinuous units. Binding of human HSF1 to the discontinuous type of HSE was observed *in vitro* but was significantly inhibited *in vivo*. These results show that human HSF1 recognizes HSEs in a slightly different way than yeast HSFs and suggest that the configuration of the unit is an important determinant for HSF-HSE interactions.

*The interaction between transcription regulatory proteins and specific DNA sequences is the obligatory first step for the regulation of gene expression. The binding of regulatory proteins is determined primarily by whether a cis-acting sequence matches the consensus recognition motif. The heat shock transcription factor (HSF), a protein evolutionarily conserved from yeast to humans, binds to the heat shock element (HSE) of target genes and regulates transcription under normal physiological conditions as well as during various stress conditions, including heat shock. HSF proteins consist of discrete functional domains, including a DNA-binding domain (DBD), a hydrophobic repeat region (HR-A/B) necessary for homotrimer formation, and a transcription activation domain at the C terminus. Homotrimerization of HSFs correlates with the binding of the DBD of each monomer to the 5-bp sequence, 5′-nGAAn-3′, in the target DNA, indicating that the HSE is composed of at least three copies of the pentanucleotide unit (1–5).

Analyses of genes targeted by the HSF of the yeast *Saccharomyces cerevisiae* (ScHsf1) have revealed that the configuration of the nGAAn unit is a critical determinant for transcriptional activation (6–19). The differently arranged nGAAn units are classified as follows: the perfect-type HSE consists of continuous inverted repeats of the unit (nTTCnnGAAnTTCn), the gap-type contains one gap between the units (nTTCnnGAAn(5 bp)nGAAn), and the step-type contains two gaps (nTTCn(5 bp)nTTCn(5 bp)nTTCn) (20). Various ScHsf1 domains are involved in HSE architecture-specific transcription. Several mutations in the DBD selectively affect transcription of the *CUP1* gene, which contains a gap-type HSE (21–23). The C-terminal activation domain termed AR2/CTA/CAD, but not the ScHsf1-specific N-terminal activation domain termed AR1/NTA/AAD, is necessary for heat- and menadione-induced transcription of *CUP1* (10, 14, 24). Deletion of AR2 also abrogates the heat shock response of several other genes containing gap-type HSEs (12). The HR-A/B region is necessary for stress-induced hyperphosphorylation of ScHsf1 and for its binding to and transcriptional activation via HSEs with three nGAAn units but not with four or more units (19). The hyperphosphorylation is regulated by regulatory domains specific to the yeast HSFs CE2 (conserved element 2) and CTM (C-terminal modulator) and is involved in the activator function of a single ScHsf1 trimer bound to the three units (16, 17).

Although ScHsf1 is essential for cell viability, this function is maintained when ScHsf1 is replaced by HSFs of the yeast *Kluyveromyces lactis*, the tomato *Lycopersicon peruvianum*, and mammalian HSF isoforms, implying that HSFs are functionally conserved among eukaryotes (25–28). A genome-wide ScHsf1 binding analysis indicates that ScHsf1 binds to the 5′-upstream regions of a set of genes that participate in a broad range of biological functions (29). The heat shock-induced transcription of many of these genes is inhibited in cells containing an *Schs1* mutation, although several of the target genes lack an apparent HSE in their promoter regions (20). In the present study, we identified the ScHsf1-binding sequences in...*
most of the target genes examined and showed that ScHsf1 binds to and activates transcription via HSEs that diverge from the canonical sequence. To explore whether the various HSE types are commonly recognized as cis-acting sequences by HSFs of other organisms, we analyzed HSE structure-specific transcriptional regulation in S. cerevisiae cells by Schizosaccharomyces pombe HSF (SpHSF) and human HSF1 (hHSF1).

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—S. cerevisiae strain HS126 (MATα ade2 his3 leu2 trp1 ura3 can1 Schsf1::HIS3 YCp-URA3-ScHsf1) contains a null mutation of the chromosomal ScHsf1 gene and bears wild type ScHsf1 on pRS316 (YCp-URA3) (14, 16, 30). For construction of hsf mutants, plasmid-born hsf derivatives were introduced into HS126, and the resident YCp-URA3-ScHsf1 was evicted by streaking transformed cells on medium containing 5-fluoro-orotic acid (14). Cells were grown in rich glycol (YPD) and enriched synthetic glucose media as described previously (14).

Plasmids—Plasmids pK157 and pSK936 are YCp-TRP1-ScHsf1 and YCp-LEU2-ScHsf1, respectively (14, 16). A derivative of pK157 lacking the extra linker region of amino acids 281–332 (ScHsf1-In52Δ, plasmid pYM37) was constructed by the overlap extension PCR method (31). An expression vector, pK346 (YEp-LEU2-PADH1-TADH1), was constructed by subcloning the ADH1 promoter-terminator fragment (PADH1-TADH1) of pVT102U (32) into YEplac181 (YEp-LEU2) (33). Plasmid pK250 (YEp-LEU2-PADH1-HA3-TADH1) was a derivative of pK346 containing three copies of the influenza hemagglutinin (HA) epitope tag sequence downstream of the ADH1 promoter. The open reading frame of S. pombe hsf1+ was amplified by reverse transcription (RT)-PCR from the total RNA of S. pombe strain Y742 (34) and cloned downstream of the HA tag in pK250 to create a plasmid expressing HA-tagged SpHSF (pYM28). A plasmid expressing human HSF1 (pYT8) was constructed by cloning the hHSF1 open reading frame into pK346. To create a plasmid expressing a fusion protein of hHSF1 and hHSF2 (hHSF1–2, plasmid pYT15), the DNA fragment encoding the C-terminal half (amino acids 181–529) of hHSF1 (pYT8) was replaced with the corresponding fragment of hHSF2 (encoding amino acids 170–536) (27, 35). To create a plasmid expressing a fusion protein of hHSF1 and ScHsf1 (hHSF1-Sc, plasmid pYT14), the C-terminal half of hHSF1 was replaced with a fragment encoding amino acids 388–833 of ScHsf1. Plasmid pYT22 was a derivative of pYT14 containing the HA tag at the N terminus of hHSF1-Sc. The addition of the HA tag did not significantly alter the activity of hHSF1-Sc (see supplemental data).

To construct the PNC1-CYC1-lacZ reporter, the fragment from −380 to −280 of PNC1 was inserted into the XhoI site lying upstream of the CYC1 promoter in pLG670Z (YEp-URA3-CYC1-lacZ) (36). Point mutations were introduced into the PNC1 fragment by the megaprimer PCR method (37).

DNase I Footprint Analysis—The 5’-end-labeled DNA fragment was incubated with a recombinant ScHsf1 polypeptide (rScHsf1) containing the N-terminal 583 amino acids, treated with DNase I, and subjected to polyacrylamide gel electrophoresis and phosphorimaging, as described previously (20).

The probe fragments contained the regions from −652 to −43 of MHP1, from −613 to −309 of SIP4, from −397 to +7 of TIP1, from −554 to +4 of PBI1, from −606 to +33 of ENO2, from −649 to +23 of HSP30, from −332 to +70 of YJL144W, from −538 to −87 of AHPI1, from −500 to +64 of PNC1, from −999 to +14 of CWP1, from −510 to −189 of GRE3, and from −737 to −155 of VPS62.

RT-PCR Analysis—The relative levels of mRNA were determined by RT-PCR analysis as described previously (16, 17).

Gene Retardation Analysis—Labeled probe DNA containing the 3P-, gap-, or step-type HSE was incubated with an rScHsf1 polypeptide or cell extract and subjected to gel electrophoresis and phosphorimaging as described previously (17, 18, 20).

Chromatin Immunoprecipitation Analysis—Chromatin was prepared from nuclei as described (38, 39). In brief, yeast cells were grown in YPD medium at 28 °C to an A600 of 2.0 and were heat-shocked at 39 °C for 15 min. Cell cultures (40 ml) were treated with 1% formaldehyde for 15 min. Crosslinking was stopped with 125 mM glycine for 5 min, and the supernatant (4 ml) was again centrifuged at 25,000 × g for 30 min. The pelletted nuclei were suspended in 0.8 ml of FA low buffer (40), and chromatin was fragmented by sonication (40). Cleared chromatin solution was subjected to immunoprecipitation with an anti-ScHsf1 serum (16), anti-hHSF1 serum (StressGen Bioreagents), or anti-HA antibody (Invitrogen). DNA was prepared from immunoprecipitates and input chromatin samples and subjected to PCR analysis as described previously (19, 40). The immunoprecipitation/input ratios of the PCR products were used for comparison. The following regions were amplified by PCR: −334 to +26 of HSP78, −485 to −133 of HSP10, −449 to −130 of CRR6, −268 to +8 of SGT2, −241 to +15 of TIP1, and −211 to +83 of GAL7.

RESULTS

Binding of ScHsf1 to Target Genes That Lack an Apparent HSE—Although the heat-induced accumulation of MHP1, SIP4, and TIP1 transcripts is abrogated by a mutation in ScHsf1, the genes neither contain a consensus HSE nor have been reported to bind to ScHsf1 (20). Here we examined the interaction between these genes and an rScHsf1 polypeptide using a DNase I footprint analysis. When the promoter fragment of MHP1 was incubated with increasing amounts of rScHsf1, a region from −211 to −176 was protected against DNase I digestion (Fig. 1A). On the SIP4 promoter, rScHsf1 bound to a region from −501 to −465 that contained several DNase I hypersensitive bands and was flanked by DNase I hypersensitive bands (Fig. 1B). An rScHsf1-binding region was also identified on the TIP1 promoter from −273 to −220 (Fig. 1C).
Taking these results together with those of the previous expression analysis (20), we conclude that ScHsf1 binds to MHP1, SIP4, and TIP1 and activates their transcription in response to heat shock.

When we used a gene analysis tool to search for HSEs in additional ScHsf1 target genes, we also failed to find a consensus HSE consisting of three regularly arranged nGA(A/G)n units in 14 of 59 target genes (20). We therefore explored the sequences that mediate the binding of ScHsf1 in these 17 genes (14 genes plus MHP1, SIP4, and TIP1). As shown in Fig. 1J, the rScHsf1-binding region of MHP1 contained a sequence, cATCaGAcgTTCa, in which 8 of 9 nucleotides matched the consensus HSE (matched nucleotides are shown in bold letters). When PIB1 was subjected to the footprint analysis, rScHsf1 bound to a region containing the sequence gTTTcTg-GGaCAcTTTc (match of 8 of 9) (Fig. 1, D and J). The promoter regions of SPI1, SSE2, TMA10, YGR250C, and YLL023C contained continuous inverted repeats of the pentanucleotide units in which the sequences slightly diverged from nGAAn (Fig. 1J).

Analyses of other target genes showed that the footprinted regions of ENO2, HSP30, and YJL144W contain gap- and step-type HSEs consisting of GAA-like sequences (Fig. 1, E–G and J). It was shown previously that ScHsf1 tolerates some deviations from the canonical GAA sequence for recognition and transcriptional activation (11, 13). Therefore, the GAA-like sequences of these 10 genes could constitute the perfect-, gap-, and step-type HSEs for transcriptional regulation by ScHsf1.

rScHsf1 also bound to the promoter regions of AHP1 and PNC1.
Cyc1 promoter—represented by Cyc1 were heat-shocked at 39 °C for 15 min, the levels of the reporter gene (lacZ) containing the Cyc1-lacZ was observed on the 5′-flanking regions of the remaining genes, Cwp1, Gre3, and Vps62, under the experimental conditions we used. It is likely that binding of ScHsf1 to these three genes is stabilized in yeast cells by interactions with other heat-induced transcription factors such as Msn2, Msn4, and Rim1 (41, 42). In total, we were able to assign ScHsf1-binding sequences in 14 of 17 genes.

The ScHsf1-binding Sequence of Pnc1 Mediates Heat-induced Transcription—We analyzed the ScHsf1-binding region of Pnc1 using a Pnc1-Cyc1-lacZ reporter gene, which contains the Pnc1 fragment from −380 to −280 upstream of the Cyc1 promoter-lacZ fusion (Fig. 2). When cells grown at 28 °C for 15 min, the levels of Pnc1-Cyc1-lacZ mRNA increased 15-fold, as shown with an RT-PCR analysis. To explore which units were necessary for the heat shock response, we changed five GAAAn units to TTTCn units (represented by unit numbers 1–5 in Fig. 2) to unrelated sequences. Altering unit 1 reduced transcriptional activation of the reporter gene (Pnc1m1-Cyc1-lacZ). Altering unit 3, which is separated from unit 1 by a regular 5-bp gap, caused a substantial decrease in lacZ mRNA (Pnc1m3-Cyc1-lacZ). The other units, especially the inverted unit 2, were necessary for efficient activation (Pnc1m2-Cyc1-lacZ, Pnc1m4-Cyc1-lacZ, and Pnc1m5-Cyc1-lacZ). Unit 2 overlaps with unit 3, and they are too close to be bound simultaneously by two ScHsf1 DBDs; the same holds true for units 4 and 5. We suggest that, on the Pnc1 promoter, the three DBDs of ScHsf1 trimer dissociate from one set of units (for example units 1, 3, and 5) and quickly rebind another set (for example units 1, 2, and 4), thereby stabilizing the protein-DNA interaction.

Although it remains possible that some GAA-like sequences are necessary for transcriptional activation, these results suggest that two directly repeating nGAAAn units separated by 5 bp are important for heat-induced transcription and that irregularly arranged units have a stimulatory role. The ScHsf1 binding regions of Ahp1, Sip4, and Tip1 also contained a pair of direct repeats and several irregular units (see Fig. 1); we therefore named the ScHsf1-binding sequence of these genes the direct repeat (DR)-type HSE.

Transcriptional Regulation by S. pombe Hsf in S. cerevisiae Cells—Although the amino acid sequences of the DBD and HR-A/B are well conserved, the structures of other regions, including the lengths of the open reading frames, vary among eukaryotic HSFs (Fig. 3A). We examined whether HSFs of other organisms also regulate the heat shock response of genes containing various HSE types. S. cerevisiae cells expressing HA-tagged S. pombe Hsf (HA-SpHsf) instead of ScHsf1 were able to grow at normal (28 °C) and elevated (37 °C) temperatures (Fig. 3B). In wild type ScHsf1 cells, a temperature shift from 28 to 39 °C caused accumulation of transcripts from genes containing HSEs with perfect repeats of four (4P-type; Hsp42, Hsp78, and Kar2) or three (3P-type; Aap1, Sef1, and Hsp10) nGAAAn units or discontinuous repeats of the gap-type (Cpr6, Cup1, and Hsp82), step-type (Fsh1, Sgt2, and Ssa3), or DR-type (Ahp1 and Tip1) (Fig. 3C). Note that the heat shock response of these genes is regulated mainly by ScHsf1, as judged from the inefficient accumulation of their transcripts in Schsf1 mutant cells (17–20). In cells expressing HA-SpHsf, transcription of all genes analyzed was activated to a level roughly equal to that of the ScHsf1 control.

The above observation implies that the regions not conserved between ScHsf1 and SpHsf are dispensable for transcriptional regulation. Consistent with this view, deletion of the AR1 activation domain from ScHsf1 does not greatly affect the activation of various target genes (14, 16). The linker region connecting the DBD and HR-A/B of ScHsf1 does not greatly affect the activation of various target genes (14, 16). The linker region connecting the DBD and HR-A/B of ScHsf1 consists of two discrete regions: the N-terminal 21 amino acids that are conserved among various HSFs; and the C-terminal extra 52 residues that are not found in other HSFs, including SpHsf. The conserved linker region is indispensable for growth of S. cerevisiae cells, whereas the extra region is not necessary for essential in vivo functions or for the structural integrity of ScHsf1 (43). In ScHsf1−/−Δ cells that express ScHsf1 lacking the extra linker region, the heat shock response of all genes analyzed was regulated normally, as in wild type ScHsf1 cells (Fig. 3C).

Transcriptional Regulation by Human Hsf1 in S. cerevisiae Cells—hHSF1 is unable to substitute for the cell viability functions of ScHsf1 because its trimer formation is inhibited by a C-terminal hydrophobic repeat region termed HR-C (27). Substituting the C-terminal region with the corresponding region of hHSF2 (hHSF1−2) allows the fusion protein to trimerize constitutively and to support the growth of S. cerevisiae cells.
Transcriptional Regulation via Various HSEs

This limited transcriptional activation by hHSF1–2 may have occurred because the fusion protein did not respond well to heat shock. The C-terminal region of hHSF1 was therefore replaced with the corresponding region of ScHsf1, which contains the CE2 and CTM regulatory regions and the AR2 activation domain (Fig. 3A). The fusion protein hHSF1-Sc supported growth at temperatures up to 33 °C, suggesting improved activity over that of hHSF1–2 (Fig. 3B). The hHSF1-Sc fusion induced the heat shock response of a subset of genes that contained the 4P- and 3P-type HSEs. However, genes containing HSEs with the discontinuous repeats of the gap-, step-, and DR-types remained unactivated (Fig. 3C).

In Vivo and in Vitro Binding of HSFs to Various HSEs—To explore the in vivo binding of HSFs to target genes, we used a chromatin immunoprecipitation analysis. The anti-Schsf1 serum precipitated promoter fragments containing 4P (HSP78)-, 3P (HSP10)-, gap (SGT2)-, and DR (TIP1)-type HSEs (but not the control GAL7 promoter, which does not contain the HSE) from an extract of Schsf1 cells grown at 28 °C (Fig. 4A). Heat shock induced the binding of Schsf1 to target promoters, as judged by a moderate increase in the amount of fragments precipitated. In extracts of control and heat-shocked hHSF1-Sc cells, the CPR6, SGT2, and TIP1 promoters were less efficiently precipitated than the HSP78 and HSP10 promoters. Similar results were obtained when extracts of cells expressing HA-tagged hHSF1-Sc were used for immunoprecipitation with an anti-HA antibody. In addition, HA-SpHSF was able to bind various HSE types in a heat-inducible manner. This is consistent with the previous observation that, unlike Schsf1, SpHSF needs heat shock to bind to the HSE (34, 44).

We next analyzed the in vitro protein-DNA interaction using the gel retardation technique. The rScHsf1 polypeptide formed protein-DNA complexes with oligonucleotides of synthetic 3P-, gap-, and step-type HSEs, and the HSE type did not significantly affect the binding affinity (upper panel of Fig. 4B). The incubation of hHSF1-Sc cell extracts with the HSE3P probe caused formation of a complex that migrated more slowly than the rScHsf1-HSE3P complex, because the expected molecular weight of hHSF1-Sc is larger than rScHsf1. Importantly, the binding affinities of hHSF1-Sc to gap- and step-type HSEs were more than 5- and 10-fold lower, respectively, than that to the 3P-type HSE. These results are consistent with those of the in vivo binding analysis and show that, unlike Schsf1, a fusion protein containing the hHSF1 DBD exhibits lower binding affinity to HSEs with the discontinuous units.

However, in the in vivo experiments, hHSF1-Sc was overexpressed. To better compare the in vivo and in vitro results, we used the same amounts of extracts from Schsf1 and hHSF1-Sc cells in the gel retardation assay. In these conditions, hHSF1-Sc generated much larger amounts of complexes with the 3P-, gap-, and even step-type HSEs than the Schsf1 control (lower panel of Fig. 4B). The lower binding affinity of hHSF1-Sc to the discontinuous HSE is therefore not enough to explain the inefficient in vivo binding to genes containing this type of HSE, suggesting that the interaction between hHSF1-Sc and HSEs is inhibited in yeast cells.

(27). The hHSF1–2 fusion exhibits the same DNA-binding specificity as hHSF1 (27, 35). Here, cells expressing hHSF1–2 were able to grow at 28 °C but not at 33 °C (Fig. 3B), and transcripts of HSP42, HSP78, and KAR2, which contain the 4P-type HSE, were increased in response to heat shock (Fig. 3C). The mRNA levels of genes containing the 3P-type HSE were moderately increased relative to those of the control, ACT1, because ACT1 mRNA slightly but reproducibly decreased at 39 °C. However, hHSF1–2 was almost completely unable to activate genes with gap-, step-, and DR-type HSEs.
We have identified ScHsf1-binding sequences in 59 genes among 62 target genes of S. cerevisiae Hsf1 (Fig. 5A). Approximately half of the target genes (26 of 59 genes) contain gap-, step-, and DR-type HSEs, indicating that sequence motifs containing discontinuous nGAAn repeats are not exceptional but are used generally as ScHsf1-binding sequences. Consistent with this, ScHsf1 bound to the gap- and step-type HSEs with similar affinity as to the 3P-type HSE in a gel retardation analysis. Among the 198 pentanucleotide units constituting the HSEs of the 59 target genes, the central trinucleotide, GAA, is well conserved. In addition, “A” is the most common nucleotide in the first position, and the fifth position appears to be random (Fig. 5B). The nucleotide frequency in the pentanucleotide units of authentic ScHsf1 targets is consistent with previous analyses of in vitro binding of ScHsf1 (45); in vitro binding of HSFs from Drosophila (45), mouse (35, 46), and human (47); in vivo binding of hHSF1 (48); and the crystal structure of the K. lactis HSF DBD complexed with DNA (49). The identification of the HSEs in almost all ScHsf1 target genes will provide important information for understanding the stress responsive transcriptional networks governed by ScHsf1 and other transcription factors. Furthermore, analysis of S. cerevisiae cells that expressed S. pombe HSF and human HSF1 showed the nGAAn configuration-dependent functioning of HSFs and the complexities of HSF–HSE interactions.

The target genes AHP1, PNC1, SIP4, and TIP1 contain a direct repeat of the nGAAn units separated by 5 bp, but the regularly positioned third unit is absent. In the PNC1 promoter, the heat shock response requires not only the directly repeated units but also several units that are located in varying positions. An exceptional HSE was also found in the MDJ1 promoter, in which the third nGAAn unit is far away from the two regularly arranged units (15). It was shown that a pair of direct repeats is not sufficient to function as the HSE (15, 20). Thus, although the presence of a regularly positioned third unit is needed for the binding of ScHsf1, functional HSEs tolerate slightly divergent configurations.

S. pombe HSF supported the growth of S. cerevisiae cells at elevated temperatures and properly regulated the heat shock response of all ScHsf1 target genes analyzed, indicating that SpHSF can bind to and activate transcription via different HSE types, as ScHsf1 does. The binding likely requires the regions conserved between the two HSFs, including the DBD, 21-amino acid linker, and HR-A/B region, whereas activation is likely to be supported by the yeast-specific regulatory domains CE2 and CTM (16). We assume that the S. pombe genome, like the S. cerevisiae genome, contains divergent HSEs that are regulated by SpHSF. Supporting this, in S. pombe cells SpHSF binds to and activates transcription of the sps1 + gene (encoding mitochondrial HSP70) that contains only a pair of inverted nGAAn units (34, 50).

In contrast to the results with S. pombe HSF, a fusion protein containing the DBD of human HSF1 was not able to bind to or...
transcriptionally activate genes containing HSEs with the discontinuous nGAAn units of the gap-, step-, and DR-types in *S. cerevisiae* cells. It has previously been reported that hHSF1 is able to mediate the heat shock response of *CUP1* and *SSA3*, which contain discontinuous HSEs (27). However, the activation levels of these genes were significantly lower than that in the SchSfs1 control (Fig. 3C). hHSF1-Sc exhibited lower binding affinity to discontinuous HSEs in the gel retardation analysis (Fig. 4B), and similar results were obtained with wild type hHSF1 synthesized by an *in vitro* transcription/translation reaction (data not shown). However, sufficiently high expression of hHSF1-Sc led to binding with this type of HSE, implying that the observed *in vitro* binding defect cannot be explained merely by the *in vitro* binding affinity. The interaction between transcription factors and recognition sequences is affected by the nucleosomal structure (51). SchSfs1 is capable of binding nucleosomal DNA in *S. cerevisiae* cells (52–54). In contrast, the *hsp70* promoter in *Hsf1*/*H11002* mouse fibroblasts is clear of nucleosomes (55), implying that mammalian HSF1 does not need to invade nucleosomes. Indeed, hHSF1 consistently fails to bind nucleosomal DNA *in vitro* (56). We suggest that, when expressed at high levels, hHSF1-Sc binds to the high affinity, continuous repeats even in the presence of nucleosomes but that this is not sufficient for binding to the low affinity, discontinuous repeats.

The reason the yeast HSFs and hHSF1 exhibited different binding affinities to the HSEs remains to be elucidated. It has been shown that binding of SchSfs1 to the gap-type HSE of *CUP1* is enhanced by amino acid substitutions in the DBD: valine to alanine at amino acid 203 and arginine to serine at amino acid 206 (21–23). These substitutions are located at or near the interface of the DBD-DBD interaction, suggesting the importance of the interface for the HSE binding (49). However, hHSF1-Sc derivatives containing the corresponding mutations failed to activate *CUP1* transcription (see supplemental data). The DBD-DBD interaction in an hHSF1 trimer may be different from that in a SchSfs1 trimer, although the amino acid sequences of the DBDs are well conserved in two proteins. The linker region is also a critical determinant for HSF-HSE interactions (43, 57). On the other hand, the trimerization domain may not be involved in the HSE architecture-specific transcription, because an SchSfs1 derivative containing a heterologous dimerization domain instead of the HR-A/B trimerization domain mediates the stress response of almost all target genes (19).

In *S. cerevisiae* and *S. pombe* cells, an HSE encoded by a single gene regulates the transcription of genes containing divergent HSEs in response to various stresses. In contrast, mammalian cells contain three HSF isoforms, HSF1, HSF2, and HSF4, which exhibit differences in tissue distribution and in stress response, suggesting that they regulate the expression of distinct genes (58–61). The different binding specificities of the isoforms to the HSEs leads to their target specificities (35, 58, 62, 63), and it remains possible that HSF2 and HSF4 recognize discontinuous nGAAn units as HSEs *in vivo*. We suggest that divergent HSEs account for differential gene- and stress-specific transcription by eukaryotic HSFs.

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