Dissection of the regulatory mechanism of a heat-shock responsive promoter in Haloarchaea: a new paradigm for general transcription factor directed archaeal gene regulation

Qiuhe Lu1,2, Jing Han1,2, Ligang Zhou1,2, James A. Coker3, Priya DasSarma3, Shiladitya DasSarma3 and Hua Xiang1,*

1State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, 2Graduate University of Chinese Academy of Sciences, Beijing, People’s Republic of China and 3University of Maryland Biotechnology Institute, Center of Marine Biotechnology, Baltimore, MD 21202, USA

Received December 6, 2007; Revised and Accepted March 18, 2008

ABSTRACT

Multiple general transcription factors (GTFs), TBP and TFB, are present in many haloarchaea, and are deemed to accomplish global gene regulation. However, details and the role of GTF-directed transcriptional regulation in stress response are still not clear. Here, we report a comprehensive investigation of the regulatory mechanism of a heat-induced gene (hsp5) from Halobacterium salinarum. We demonstrated by mutation analysis that the sequences 5′ and 3′ to the core elements (TATA box and BRE) of the hsp5 promoter (P_hsp5) did not significantly affect the basal and heat-induced gene expression, as long as the transcription initiation site was not altered. Moreover, the BRE and TATA box of P_hsp5 were sufficient to render a nonheat-responsive promoter heat-inducible, in both Haloferax volcanii and Halobacterium sp. NRC-1. DNA–protein interactions revealed that two heat-inducible GTFs, TFB2 from H. volcanii and TFBb from Halobacterium sp. NRC-1, could specifically bind to P_hsp5 likely in a temperature-dependent manner. Taken together, the heat-responsiveness of P_hsp5 was mainly ascribed to the core promoter elements that were efficiently recognized by specific heat-induced GTFs at elevated temperature, thus providing a new paradigm for GTF-directed gene regulation in the domain of Archaea.

INTRODUCTION

Archaea are prokaryotic microorganisms similar to bacteria in many aspects of morphology and metabolism, but are more closely related to eukarya in the genetic information processing system (1,2). The archaeal basal transcription machinery is fundamentally related to the core components of the eukaryotic RNA polymerase (RNAP) II apparatus, possessing a multi-subunit RNAP and two general transcription factors (GTFs). These GTFs, termed TBP and TFB, are homologues of the eukaryal TATA-box binding protein and transcription factor IIB (TFIIB), respectively (3,4). In the process of transcription initiation, TBP first recognizes and binds to the TATA box, resulting in bending of DNA at the promoter region. Then TFB binds to the TBP–DNA complex, making sequence-specific contact with the BRE (TFB recognition element) upstream of the TATA box. This contact directs RNAP to the promoter, thus specifically initiating transcription at an initiator sequence located about 25 bp downstream of the TATA box (5).

Intriguingly, although the archaeal transcription apparatus is eukaryotic-like, many putative transcription regulators encoded by archaea are homologous to those in bacteria (6). Several instances of negative control of archaeal transcription by such regulators have been described. The metal-dependent repressor 1 (MDR1) from Archaeoglobus fulgidus (7) and LrpA from Pyrococcus furiosus (8), were found to bind to the operator sequences overlapping the transcription start sites, whereas the Lrs14 from Sulfolobus solfataricus (9,10) and TrmB from Thermococcus litoralis (11) bind to the sites overlapping the BRE/TATA elements. Thus, these regulators could inhibit transcription initiation through occlusion of RNAP or TBP–TFB recruitment. On the other hand, there are fewer known mechanisms of positive control of archaeal transcription. GvpE, resembling eukaryal basic leucine-zipper protein, has been identified as an activator in the gas vesicle synthesis in haloarchaea (12,13), but the exact mechanism has yet to be elucidated. One of the best
characterized archaeal transcriptional activators is Ptrl from *Methanococcus jannaschii*. It could bind to the sequences upstream of the core promoter elements of ferredoxin A (*fdxA*) and rubredoxin 2 (*rb2*) genes, and activate transcription through direct recruitment of TBP to these promoters (14). It should be mentioned that multiple TBPs and TFBs are present in several archaea, including *Halobacterium* sp. NRC-1 (15–18). This raises another possibility that particular TBP–TFB combinations may recognize different promoters and therefore regulate different genes (19). Recently, microarray-based studies have provided evidence that certain GTFs (TBPs/TFBs) interact with specific groups of promoters and are likely involved in global gene regulation (20), and TBP and TFBa co-regulate, either directly or indirectly, a subset of genes that account for over 10% of the *Halobacterium* sp. NRC-1 genome (21).

Heat-shock response is a widespread physiological phenomenon in all three domains of life and an attractive process for investigation of gene expression regulation. Current genome projects have identified numerous heat-shock proteins in archaea, such as HSP70 (DnaK), HSP60 (GroEL), HSP40 (DnaJ), GrpE and many small heat-shock proteins (sHSP) (18, 22, 23), but no homologues of *GroEL*, HSP40 (DnaJ), GrpE and many small heat-shock proteins in archaea, such as HSP70 (DnaK), HSP60 (24, 25) and HSR1 from *A. fulgidus* (26) might specifically bind to the promoters of some heat-shock genes under optimal growth temperature, and release from them in response to heat shock. Intriguingly, one of the two TFB-related genes in *P. furiosus* is transcriptionally heat-inducible, implying it may be involved in heat-shock regulation (27). For extremely halophilic archaea, Daniels and co-workers have studied a heat-shock regulatory GTF, *Ptr2* from *H. salinarum* (30), suggesting that TFB-modulated heat-shock response elements (HSE) have been identified. To date, only a few studies on heat-shock response have been reported in the domain of Archaea. Among the thermophilic archaea, it has been proposed that the Phr from *P. furiosus* (24, 25) and HSR1 from *A. fulgidus* (26) might specifically bind to the promoters of some heat-shock genes under optimal growth temperature, and release from them in response to heat shock. Intriguingly, one of the two TFB-related genes in *P. furiosus* is transcriptionally heat-inducible, implying it may be involved in heat-shock regulation (27).

### MATERIALS AND METHODS

#### Strains, plasmids and primers

*Escherichia coli* JM109 was used as a host for the cloning experiments and *E. coli* BL21 (DE3) (Novagen, Madison, WI, USA) for over expression of recombinant proteins. All *E. coli* strains were grown in Luria–Bertani (LB) medium at 37°C (31). When needed, ampicillin and kanamycin were added to a concentration of 100 and 50 μg/ml, respectively. Unless otherwise noted, *H. salinarum* CGMCC 1.1959, *Halobacterium* sp. NRC-1 and *H. volcanii* DS70 (32) were cultivated at 37°C in CM medium (per liter, 7.5 g Bacto casamino acids, 10 g yeast extract, 3.0 g trisodium citrate, 200 g NaCl, 20 g MgSO₄·7H₂O, 2.0 g KCl, 50 mg FeSO₄·4H₂O and 0.36 mg MnCl₂·4H₂O, pH 7.2). When required, mevinolin was added to a concentration of 5 or 10 μg/ml for *H. volcanii* or *Halobacterium* sp. NRC-1, respectively. The plasmid pNP22 (33) was used as the source for *bga* gene, while the *H. volcanii*–*E. coli* shuttle vector pWL102 (34) was used for constructing the *bga* reporter module. The primers used in this study are listed in Table 1.

#### Cloning the hsp5 gene from *H. salinarum* CGMCC 1.1959

Using the sequence information of the hsp5 gene (VNG_6201G, in GenBank AE004438) of *Halobacterium* sp. NRC-1 (18), primers hspF52 and hsp5R were designed to amplify the corresponding gene of *H. salinarum* CGMCC 1.1959 and its promoter region. The hspF52 primer located 101 bp upstream of the hsp5 start codon, while hsp5R was complementary to an 18 bp DNA region in the 3′ terminus of the hsp5 open reading frame (ORF). The resulting PCR product was ligated into the vector pUCm-T (Sangon, China) and sequenced.

#### Constructs used for transformation of haloarchaea and reporter gene analysis

For analysis of P*_{hsp5}* activity in vivo, we used a plasmid-based transcriptional reporter system as described previously (33, 35). The P*_{hsp5}* region was amplified by PCR using primers hspF82 and hspRNdeI, with CGMCC 1.1959 genomic DNA as template. The primer hspRNdeI was complementary to a DNA region including the first 1.1959 genomic DNA as template. The primer hspRNdeI was complementary to a DNA region including the first 3′-terminus of the hsp5 gene, while hsp5R was complementary to an 18 bp DNA region in the 3′ terminus of the hsp5 open reading frame (ORF). The resulting PCR product was ligated into the vector pUCm-T (Sangon, China) and sequenced.

In this study, we report a comprehensive investigation of transcriptional control of the hsp5 gene that encodes a sHSP in *Halobacterium*. Using in-depth genetic and biochemical approaches, we demonstrated, for the first time, that alternative GTFs, rather than bacterial-type regulators, specifically modulated the heat-shock inducibility of the hsp5 promoter in both *H. volcanii* and *Halobacterium* cells. Therefore, our results establish a new paradigm of GTF-modulated transcriptional regulation in the domain of Archaea.
specific forward primers carrying the desired mutated nucleotides (FM1-FM12, Table 1), and the reverse primer bgaHRNcoI were used to amplify the \( P_{hsp5} \)-bgaH fusion fragments from pL37. The resulting PCR products were inserted into pWL102 to generate the desired constructs. Similarly, the \( P_{bop} \)-bgaH fusion was generated by PCR amplification using the primers bopF and bgaHRNcoI with the plasmid pNP22 as the template. To generate \( P_{bop} \)-\( P_{hsp5} \) chimeras (bBhsp, bThsp and bBTbop), primers containing the BRE or/and TATA box sequence of \( P_{bop} \) (Table 1) were used with plasmid pL37 as the PCR template. The \( P_{hsp5} \)-bgaH fusion were cloned into pWL102 at BamHI and NcoI sites. The fidelity of PCR-amplified products in these recombinant plasmids was confirmed by DNA sequencing.

**Table 1. Primers used in this study**

| Name       | Sequence (5'-3')<sup>a</sup> |
|------------|-------------------------------|
| hspF82     | CTAGGATCCCGACGACCTCATGTA      |
| hsp5R      | TACGCGTGCAGCTCGAT             |
| hspRNdI    | ACTCATATGAGAGCTGGGATCTATCA    |
| hspF52     | GCGGGATCCATTTTTTGTTTGTAGAGAAGATTTTTTTA |
| hspF42     | GCGGGATCCATTTTTTGTTTGTAGAGAAGATTTTTTTA |
| hspF37     | GCGGGATCCAATTCTTTACGGAGATATTTAG |
| hspF32     | GCGGGATCCAATTCTTTACGGAGATATTTAG |
| hspF24     | GCGGGATCCCAAATCGCCGACATAGTTTT |
| hspF22     | ACTGTTCCAACCGGTCGGC           |
| hspR22     | TGGGTGTCCGCTGCTACTTC          |
| bgaH_F2    | TCAGGGAAATTCGGTAGTGA          |
| bgaH_R2    | AGGTTCCGCAAACGCTCTCA         |
| 7SF        | CCAACGTTGAGAGCTGC            |
| 7SR        | GTTGGTGTCCGCTGCTACTTC        |
| bgaHRNcoI  | GACCATCGTTACGCGAGATCTCC      |
| bgaHRNcoI  | GACCATCGTTACGCGAGATCTCC      |
| hsp5seq    | GCACTACGGCTCGGAGAGG           |
| hgaSeq     | AGCCTCGGCACCTCTGACTGA         |
| FM1        | GCGGGATCCAGAAATTTTTCAGGGAATGGAC |
| FM2        | GCGGGATCCAGAAATTTTTCAGGGAATGGAC |
| FM3        | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| FM4        | GCGGGATCCAGAAATTTTTCAGGGAATGGCAATGTTTTG |
| FM5        | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| FM6        | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| FM7        | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| FM8        | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| FM9        | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| FM10       | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| FNM10      | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| FM11       | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| FM12       | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| hspR10     | ACTGATTGCTGTATTACACGCAATTC    |
| bopF       | GCGGGATTCGCTGATAGTTACACACATCC |
| hBbopF     | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| hTBopF     | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| bThspF     | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| bBThspF    | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| boppF      | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| boppR      | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| tfbBF      | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| tfbBR      | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| tfb2F      | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |
| tfb2R      | GCGGGATCCAGAAATTTTTTACGAAATGGCAATGTTTTG |

<sup>a</sup>Restriction sites are underlined; the substituted nucleotides in primers FM1 to FM12 are indicated by bold characters.

Isolation of RNA from cells under heat shock

Cells of *Halobacterium* sp. NRC-1, *H. salinarum* CGMCC 1.1959 or *H. volcanii* were grown at 37°C until mid-logarithmic growth phase, and then shifted to elevated temperatures (45, 48, 55 or 58°C) for heat shock for 15 min. The heat-shocked cells (5 ml) were immediately collected for RNA extraction using TRIzol reagent (Gibco BRL, Gaithersburg, MD, USA) according to the manufacturer’s instructions, with cells remaining at 37°C as the controls.
Northern blot and primer extension analyses

Activities of all the promoters in this study were measured by northern blot analysis. For monitoring the gene expression of hsp5, bgaH and bop, the hsp5 probe (228 bp), bgaH probe (340 bp) and bop probe (341 bp) were amplified with primer pairs hspF/hspR, bgaH/F/bgaHpR and boppF/bopR (Table 1), respectively. The 7S RNA was monitored as an internal control by a specific probe (110 bp) amplified with the primers 7SF and 7SR (Table 1). All the PCR products used for probes were labeled with [γ-32P]-dCTP and subjected to northern blot analysis as described previously (37). The northern hybridization signal of the hsp5 or reporter gene (bgaH) was quantified using Quantity One software (Bio-Rad, Hercules, CA, USA) by scanning the exposed X-ray films, and normalized against the signal of the internal control (7S RNA). Heat-shock induction folds were determined by taking the ratio of the normalized heat-shock to nonshock hsp5 or bgaH signals. Quantification of these transcript levels and heat-shock induction folds were based on the results of two or more independent experiments for each promoter.

To determine the transcriptional start sites of the P_hsp5-controlled hsp5 in CGMCC 1.1959 and bgaH reporter gene in H. volcanii, the primer hsp5seq hybridizing to 20 nt within the hsp5 gene and the primer bgaHseq complementary to a 20 bp DNA region within the bgaH gene were used. These primers were labeled at the 5′-end with [γ-32P]-ATP and were used for both DNA sequencing and primer extension as previously described (37).

Overexpression and purification of TFBs

The tfbB and tfbG genes were cloned from Halobacterium sp. NRC-1 by PCR with primer pairs tfbBF/tfbBR and tfbGF/tfbGR, respectively, and the tfb2 gene was amplified from H. volcanii with primers tfb2F and tfb2R (Table 1). All the PCR fragments were sequenced and cloned into the expression vector pET28a at the BamHI/HindIII sites. The recombinant plasmids were then introduced into E. coli BL21 (DE3). The E. coli recombinants were cultured until mid-logarithmic phase and then induced with 1 mM IPTG for an additional 4 h. All the histidine-tagged proteins were purified by a Ni-NTA agarose column (Novagen) according to the manufacturer’s instructions. The eluted solution containing TFB was identified by SDS–PAGE and subsequently pooled and dialyzed against buffer A [50 mM Tris–HCl (pH 8.0), 1 mM EDTA, 10% glycerol, 0.1 mM ZnCl2, 50 mM MgCl2, 2 M KCl, and 0.2 g/l of PMSF] and subsequently concentrated by ultrafiltration using an Amicon Ultra-15 centrifugal filter device with 10 kDa molecular-weight cutoff (Millipore, Bedford, MA, USA). The concentrations of purified proteins were determined by using the BCA™ protein assay kit (Pierce, Rockford, IL, USA).

Determination of the interaction between P_hsp5 and TFBs by EMSA

In order to test the specificity of DNA binding of TFB2, TFBb and TFBg, the following DNA fragments were prepared for EMSA: FW (−37 to +10 region of the wild-type P_hsp5), FM (BRE and TATA box of P_hsp5 in FW were replaced by corresponding parts of P_bop) and FD (BRE and TATA box of P_hsp5 in FW were deleted) were generated by PCR, using [γ-32P]-ATP-labeled primer pairs hspF37/hspR10, bBThspF/hspR10 and hspF24/hspR10 (Table 1), respectively. These [γ-32P]-ATP-labeled PCR products were purified with UNIQ-10 Column (Sangon, China). Interaction between P_hsp5 and TFBs was performed as described by Ken and Hackett (38) with minor modifications. Briefly, TFBs (0–4 μM) were incubated with 20 fmol 32P-labeled DNA in a 20 μl reaction mixture containing 0.5 M NaCl, 25 mM EDTA and 3 μg poly (dI/dC) at 37 or 50°C for 30 min. The resulting complexes were run on a 5% polyacrylamide gel (acylamide/bisacrylamide weight ratio of 60 : 1) in 100 mM sodium phosphate buffer (pH 6.0, preheated to 37 or 50°C). The gels were electrophoresed at 3 V/cm for 5–6 h.

RESULTS

Cloning and transcriptional analysis of hsp5 gene in H. salinarum

It has been reported that the hsp5 is one of the most highly upregulated genes under heat shock in Halobacterium sp. NRC-1 (39, 40). To determine whether the corresponding gene was also present and heat shock-inducible in some other Halobacterium strains, we have cloned the hsp5 gene and its promoter region from the genome of H. salinarum CGMCC 1.1959. Interestingly, pairwise sequence comparisons showed that hsp5 of CGMCC 1.1959 exhibited 100% identity with that of Halobacterium sp. NRC-1. Moreover, when the CGMCC 1.1959 cells were grown to the mid-logarithmic phase at 37°C and then shifted to elevated temperatures (45, 48, 55 or 58°C) for 15 min, northern blotting clearly revealed that the hsp5 transcripts increased upon temperature rising (up to ~12-fold at 58°C), exhibiting a typical pattern of heat-shock response (Figure 1A).

The transcription initiation site of hsp5 was then demonstrated by primer extension. Under both normal growth temperature and heat-shock conditions, the hsp5 transcripts were initiated from the same residue (G) located 19 bp upstream of the ATG start codon (Figures 1B and 2A). Further analysis of the hsp5 promoter (P_hsp5) sequence identified a typical TATA box (−31 TTTTTTA −25) located 25 bp upstream of the transcription initiation site, and a putative BRE (−37 AGAAAA −32) immediately upstream of the TATA box (Figure 2A). Interestingly, just 2 bp upstream of these putative core promoter elements, there was the stop codon (−41 TGA −39) of the upstream gene. To ascertain if any regulatory elements exist adjacent the BRE/TATA box, we defined the DNA sequence from −82 to +19 as the full-length promoter region (Figure 2A) for investigation of expression regulation.
mapping the 5' boundary of the P \textit{hsp}5 by deletion analysis

To determine the minimal region of the promoter P \textit{hsp}5 for both basal and heat-inducible function, we created a set of promoters with different 5'-deletions (from \textit{−82} to \textit{−32}) and the same 3' terminus (\textit{+28} within the \textit{hsp}5 coding region) by PCR amplification (Figures 2A and 3A). The full-length and shortened promoters fused with \textit{bga}H gene were cloned into plasmid pWL102. These constructs, named pL82, pL52, pL42, pL37 and pL32 (Figure 3A), respectively, were introduced into \textit{H. volcanii}. The relative activity of each promoter was tested under both normal growth temperature (37°C) and heat shock (58°C), by measuring the levels of \textit{bga}H transcripts. It was revealed that the full-length promoter in pL82, and 5' flanking-shortened mutants in pL52, pL42 and pL37 exhibited similar transcription activities, with about 8 to 11-fold upregulation under heat shock (Figure 3), resembling the native promoter in \textit{H. salinarum} CGMCC 1.1959. However, when the 5'-end of \textit{P \textit{hsp}5} was shortened to \textit{−32} bp where the putative BRE (\textit{−37} AGAAAA \textit{−32}) was deleted, both the basal and heat-induced transcription activities became hardly detectable (Figure 3B). Moreover, the putative TATA box (\textit{−31} TTTTTTA \textit{−25}) was also extremely important. Substituting three of the six nucleotide ‘T’ with ‘G’ made the promoter completely inactive (data not shown). These results demonstrated that the 5' terminus of the functional \textit{P \textit{hsp}5} extends to the position \textit{−37}, which was exactly the 5' boundary of the core promoter elements, the BRE and TATA box.

Mutational analysis of the sequence downstream of the
TATA box in \textit{P \textit{hsp}5}

Since the sequence upstream of the BRE and TATA box was not involved in the transcriptional regulation of the \textit{P \textit{hsp}5}-controlled genes, we then analyzed to determine whether the downstream sequence accounted for the heat-shock response. PCR-based scanning mutagenesis was performed to alter the targeted nucleotides downstream of the TATA box. The resulting mutants (M1 to M12, and Mdel), based on pL37, were introduced into \textit{H. volcanii}. The transcription efficiency of each mutated promoter was determined by northern blot analysis, and was compared with that of the intact functional promoter in pL37. It was shown that both basal transcription and heat induction (12 ± 4 fold) were not significantly changed for these
mutated promoters, except for mutant M10 that completely lost transcriptional activity (Figure 4). Further analysis of the mutations within M10 revealed that the transcription initiation point was altered; thereby, the transcription initiation was inhibited. When the transcription initiation residue (G) was restored in the mutant NM10, it acquired the similar basal and heat-inducible transcription activities as the native promoter (Figure 4). Noteworthily, there was a large inverted repeat (IR) sequence (-5 TGGCT-N4-TCA-N3-TGA-N2-AGCCA +20), which overlapped the transcription start site (Figure 4), and was likely to be a regulatory element for heat-shock response. However, point mutations of the 50-half (M9 to M12) or even deletion of the 30-half (Mdel) of this IR did not significantly affect the transcription activity of P_{hsp5} under either normal growth temperature or heat-shock conditions, implying that this region was not involved in heat-shock regulation in H. volcanii. These results suggested that there were likely no heat-shock response elements within the region between the core promoter elements (BRE and TATA box) and the translational start codon.

**BRE and TATA box are responsible for both basal and heat-induced transcription of P_{hsp5}**

The earlier results suggested that only the core promoter elements, the BRE and TATA box, were the likely candidates for regulation of the detectable basal as well as the strong heat-inducible transcriptional activity of P_{hsp5}. To confirm this, we constructed a set of chimeric promoters by recombining the BRE, TATA box and downstream sequences between the P_{hsp5} and a nonheat-inducible promoter of the bacterio-opsin gene (P_{bop}) (42). These promoters were then ligated with bgaH ORF and inserted into pWL102. The bop promoter (in the construct bopW) consisted of the TATA box and six upstream nucleotides (we assigned it as the putative BRE in this article) as well as the sequences between the TATA box and the bop ATG start codon, while the wild-type P_{hsp5} (in construct hspW) used the same sequence as that in pL37. The chimeric promoters bBhsp, bThsp or bBTbsp were constructed by substitution of the BRE, TATA box or both elements of the P_{hsp5} with the counterparts of P_{bop}. Similarly, the chimeric promoters hBbop, hThop or hBTbop were derived from P_{bop}, by substitution with the BRE, TATA box or both elements of the P_{hsp5} (Figure 5A).

Each of these constructs was introduced into H. volcanii for transcriptional analysis. Significantly, while P_{bop} was heat-inducible (hspW, Figure 5B) and P_{bop} was not (bopW, Figure 5C) as expected, it was clearly shown that when the BRE/TATA elements of P_{hsp5} were replaced by the counterparts of P_{bop}, it rendered the nonheat-inducible
promoter $P_{bop}$ completely heat-inducible in the resulting chimeric promoter (hBTbop, Figure 5C). On the contrary, if the BRE/TATA elements of the $P_{hsp}$ were substituted by those of $P_{bop}$, the resulting chimeric promoter bBThsp lost heat-inducible activity, and the transcript level of the reporter gene became too low to be detectable by northern blotting (Figure 5B). These results reinforced the conclusion that only the BRE and TATA elements of $P_{hsp}$ accounted for the heat-inducible feature of this promoter in *H. volcanii*.

Interestingly, it is likely that both the BRE and TATA box of the $P_{hsp}$ are heat responsive elements, since retaining either the TATA box or BRE in the chimeric promoters derived from $P_{hsp}$ (bBhsp and bThsp, Figure 5B), or substitution with either the BRE or TATA box of $P_{hsp}$ in the $P_{bop}$-derived chimeras (hBbop and hTbop, Figure 5C), the resulting chimeric promoters acquired higher transcriptional activities (~2- to 5-fold) at elevated temperature than at normal growth temperature. Thus, both the BRE and TATA box of $P_{hsp}$ are important for heat-shock response, while their combination provided the most significant contribution to transcriptional activation under heat shock (hspW and hBTbop, Figure 5B and C).

Transcriptional analysis of the chimeric promoter hBTbop in *Halobacterium* sp. NRC-1

Considering that the promoter $P_{hsp}$ was acquired from *Halobacterium* and our above investigations were mainly

---

**Figure 3.** Deletion analysis of the 5' flanking region of $P_{hsp}$. (A) Schematic representations (not to scale) of the constructs pL82, pL52, pL42, pL37 and pL32, showing $P_{hsp}$ and 5' flanking sequence-shortened promoter mutants (solid lines, −82/+28 to −32/+28) that were fused with the bgaH reporter gene (filled gray arrow). The heat-shock induction fold for each promoter determined by northern blot analysis of the bgaH signals (B) is indicated. (B) Northern blot analysis of the *H. volcanii* transformants harboring pL82, pL52, pL42, pL37 and pL32, respectively. Cellular RNAs (10 μg) were extracted from *H. volcanii* transformants under nonshock (zero time point) and heat-shock (58°C for 15 min) conditions. Hybridization signals corresponding to bgaH transcripts and 7S RNA (the internal control) are pointed.

**Figure 4.** Mutagenesis of sequence downstream of the TATA box in $P_{hsp}$. The DNA sequence of wild-type $P_{hsp}$ is given at the top (pL37), BRE (double underlined), TATA-box (single underlined), transcription initiation site (asterisk) and the translational start codon of $hsp$ (boxed) are indicated. A large IR sequence (IR1+IR2) is indicated by the opposing arrows. The mutated nucleotides of different mutants (M1–M12) are shown below the wild-type promoter sequence. Dashes in the mutant sequences indicate nucleotides that are identical to the wild-type $P_{hsp}$. The deleted sequences are indicated by the dots (Mdel). The basal (37°C) and heat-induced (58°C) transcript levels of bgaH controlled by these promoters in *H. volcanii* were determined by northern blotting. The basal transcription activities of the mutant promoters are expressed as a percentage of the wild-type activity (set as 100% wt) and the heat-shock induction folds are calculated as described in Materials and methods section.
performed in Haloferax, we then further asked whether the conclusion made sense in another model haloarchaeon Halobacterium sp. NRC-1, which is phylogenetically closely related to H. salinarum CGMCC 1.1959. First, we analyzed the mRNA levels of bop in Halobacterium sp. NRC-1, and confirmed that the bop promoter was not heat-inducible (Figure 6A). Then, the construct hBTbop was introduced into Halobacterium sp. NRC-1 and the transcript levels of the reporter gene (bgaH) under both nonshock and heat-shock conditions were determined. Our results confirmed again that the BRE and TATA box were indeed the determinants of the heat-inducible activity of P_hsp5. The chimeric promoter in hBTbop, with the BRE/TATA elements from P_hsp5 and downstream sequence from P_bop, acquired strong heat-inducible transcriptional activity (Figure 6B).

Transcriptional profiling of GTF genes of Halobacterium sp. NRC-1 in response to heat shock

Previous investigations have demonstrated that the gene encoding the transcription factor TFB2 in Haloferax is upregulated under heat shock (30). In order to determine which GTF genes in Halobacterium sp. NRC-1 were responsive to heat-shock stress (if any), we have analyzed our microarray database (39, and unpublished data). As shown in Table 2, the expression level of the six tbp genes (tbpA-F) were scarcely altered after heat shock, ranging from about 1.05 to 1.25-fold. However, among the seven tfb genes (tfbA~G), tfbB and tfbG were significantly upregulated, with fold changes of about 1.68 and 2.41, respectively. Therefore, it was of interest to determine whether these heat-induced GTFs, TFB2 from Haloferax and TFBb or TFBg from Halobacterium, were involved in transcriptional regulation of P_hsp5 by recognition of the promoter elements.
Table 2. DNA microarray analysis of transcriptional changes of the GTF genes under heat shock in Halobacterium sp. NRC-1.

| Gene ID | Gene Name | Fold change values | log₂(×) ratio | Standard deviation of log₂(×) ratio |
|---------|-----------|--------------------|---------------|-----------------------------------|
| 5039    | tbpA      | −1.024             | −0.033        | 0.018                             |
| 5052    | tpbB      | 1.253              | 0.316         | 0.173                             |
| 5142    | tpbC      | 1.096              | 0.121         | 0.178                             |
| 5163    | tpbD      | −1.050             | −0.073        | 0.306                             |
| 2243    | tpbE      | 1.029              | 0.036         | 0.171                             |
| 6438    | tpbF      | −1.005             | −0.008        | 0.109                             |
| 2184    | tpbA      | 1.197              | 0.253         | 0.136                             |
| 734     | tpbB      | 1.681              | 0.592         | 0.658                             |
| 6351    | tpbC      | 1.102              | 0.125         | 0.306                             |
| 869     | tpbD      | 1.093              | 0.113         | 0.325                             |
| 6389    | tpbE      | 1.006              | 0.008         | 0.122                             |
| 315     | tpbF      | −1.074             | −0.094        | 0.201                             |
| 254     | tpbG      | 2.412              | 1.199         | 0.450                             |

*Microarray data processing and statistical analysis were carried out as previously described (39).

TFBb and TFB2 specifically bind to hsp5 at elevated temperature

To test whether the Pₜₕ₅ was recognized by the heat-induced general transcription factors, TFB2 from *H. volcanii*, and TFBb and TFBg from *Halobacterium* sp. NRC-1, they were overproduced and purified in *E. coli*, and were subjected to electrophoretic mobility shift assay (EMSA) to determine their interactions with the Pₜₕ₅ DNA and its mutants (Figure 7). Interestingly, TFB2 could efficiently bind to the wild-type Pₜₕ₅ (FW), with even higher binding efficiency at 50°C than at 37°C, as more DNA-protein complex and less proportion of free FW DNA appeared at 50°C when same concentration of TFB2 was included in the reaction (Figure 7B). This binding appears to be specific, since interaction between TFB2 and the BRE/TATA-deleted fragment (FD) was not detectable in the same EMSA. A relatively weak interaction between TFB2 and FM (BRE/TATA of Pₜₕ₅) was detectable; however, it only occurred at 50°C when high concentrations of TFB2 (e.g. 4 μM) were available (Figure 7B). These results may help explain the heat-inducibility of Pₜₕ₅ in *Halofexax*, as the TFB2 was upregulated under heat shock (30), and could efficiently bind to Pₜₕ₅ at high temperature.

Significantly, when TFBb and TFBg were incubated with the Pₜₕ₅ DNA (FW) and Pₜₕ₅-derived mutants (FM and FD), only TFBb but not TFBg could specifically bind to the Pₜₕ₅ DNA at the high temperature (50°C), and no detectable interactions were observed for either of the TFBs at the lower temperature (37°C) (Figure 7C and D). Moreover, TFBb and TFBg could not interact with the Pₜₕ₅-derived mutants (FM and FD) in EMSA under the same conditions, suggesting that the interaction of TFBb and Pₜₕ₅ is specific and likely temperature-dependent. These results indicated that TFBb, but not TFBg, might regulate the hsp5 gene expression at elevated temperature in *Halobacterium*.

Taken together, our results have established a new paradigm for archaeal gene regulation in response to environmental changes. Under heat shock, a few heat-inducible GTFs, such as TFB2 in *Halofexax* or TFBb in *Halobacterium*, together with the corresponding TBPs, yet to be identified, could immediately modulate a group of downstream target genes, including the small heat-shock gene hsp5, to cope with the environmental stress.

**DISCUSSION**

Multiple GTFs are present in haloarchaea and have been speculated to regulate differential gene expression for years (19), and systems approach has provided supports that the GTFs in *Halobacterium* sp. NRC-1 likely accomplish large-scale regulation of transcription (20,21). However, detailed studies of the role of GTF-directed transcriptional regulation of specific genes in response to environmental signals in archaea are limited. In this article, we demonstrated that the BRE and TATA box of the Pₜₕ₅ play a critical role in both basal and heat-induced gene expression, which was confirmed by both genetic and biochemical approaches. Therefore, our work has established a new paradigm for TFB–TBP modulated gene regulation in the domain Archaea.

The hsp5 gene and its homologs, encoding sHSPs, are present in numerous haloarchaeal genomes including *Halobacterium* sp. NRC-1, *Haloarcula marismortui* and *Haloquadratum walsbyi* (15,17,18). These proteins belong to the Hsp20/α-crystallin family (43), and act as molecular chaperones to protect cellular proteins against irreversible aggregation during stress conditions (44). The hsp5 gene is upregulated under heat shock in both *Halobacterium* sp. NRC-1 (39,40) and *H. salinarum* CGMCC 1.1959 (Figure 1A), and the hsp5 promoter also exhibited similar heat-inducibility in *H. volcanii* (Figure 2B). Deletion analysis demonstrates that the 5’ boundary of the functional promoter of hsp5 is exactly at the position of the putative BRE and TATA box (Figure 3). Therefore, there is no upstream activation sequence (UAS) adjacent the BRE/TATA box in the defined full-length promoter Pₜₕ₅. It is noteworthy that there is an IR overlapping the transcription initiation site in Pₜₕ₅ (Figure 4). This IR resembles the heat-shock regulatory elements usually presented in many bacterial (45–47) and some archaeal heat-shock genes (26). For instance, a conserved palindromic motif, CTAAAC-N₅-GTTAG, located downstream of the BRE/TATA elements of the promoter Pₜₕ₅ and Pₜₕ₅² in *A. fulgidus*, is involved in heat-shock regulation by binding of the heat-shock repressor HSR1 (26). However, the IR in Pₜₕ₅ was not found to be involved in the Pₜₕ₅-controlled heat-shock response in *H. volcanii*, since mutagenesis of the sequences downstream of the TATA box including this IR did not significantly change the promoter activity, as long as the transcription initiation site was not altered (Figure 4). Moreover, replacement with the BRE/TATA box of Pₜₕ₅, rendered the nonheat-inducible promoter (Pₜₕ₅) heat-inducible, in both *H. volcanii* and *Halobacterium* sp. NRC-1 (Figures 5 and 6). Therefore, there is also no heat-shock response element downstream of the core promoter elements, and the BRE and TATA box of Pₜₕ₅ are likely the only
elements accounting for both basal of heat-inducible transcription in these haloarchaea. These results are slightly different from the earlier observations for the \( P_{cct1} \) in \( H. \ volcanii \), where the heat-responsiveness of \( P_{cct1} \) is mapped to the TATA box and surrounding sequences, including the putative BRE and two downstream sites (29). Nevertheless, it is most likely that the sequences surrounding the TATA box in \( P_{cct1} \) are also the contact sites of TFB or TBP; hence both \( P_{cct1} \) and \( P_{hsp5} \) might use the same mechanism of GTFs directed strategy in response to heat shock.

This novel strategy of gene expression regulation for \( P_{hsp5} \) was further supported by direct biochemical evidence that \( P_{hsp5} \) was recognized by specific heat-inducible GTFs, TFB2 from \( Haloferax \), and TFBb from \( Halobacterium \) (Figure 7). Our EMSA results indicated that both TFB2 and TFBb were able to recognize the corresponding core promoter without the assistance of TBPs, at least in vitro when high concentration of TFBs was supplied (Figure 7B and C). It was observed that the binding efficiency of TFB2 was likely higher than that of TFBb. Since \( H. \ volcanii \) has a lower salt optimum than \( Halobacterium \) strains and both proteins were over expressed in \( E. \ coli \), this different affinity is likely due to the presence of more properly folded molecules of TFB2, compared to TFBb, in the purified samples. The high molecular weight DNA–protein complexes appeared around the loading wells (Figure 7B) are likely the aggregation of sufficient

---

**Figure 7.** EMSA analysis of interaction between TFBs and double-stranded DNA fragments of wild-type and mutant \( P_{hsp5} \). (A) Nucleotide sequences of the fragments used in EMSA. FW, DNA fragment of wild-type \( P_{hsp5} \); FM, BRE/TATA-substituted mutant; FD, BRE/TATA-deleted mutant. The sequences from \( P_{hsp5} \) are shaded in gray; the sequences from \( P_{bop} \) are boxed; the deleted nucleotides are indicated in dash. Putative BRE (italic) and TATA-box (bold) elements of \( P_{hsp5} \) and \( P_{bop} \) are indicated. The sequences up- and downstream of the promoters generated by PCR primers are also presented. (B–D) EMSA performed on wild-type (FW) and mutant (FM and FD) \( P_{hsp5} \) DNA fragments (20 fmol) with TFB2 (B), TFBb (C) and TFBg (D) at low (37°C) or high (50°C) temperature. The amount of proteins (0–4 \( \mu \)M) in each lane is indicated. The free probes (FP) and DNA–protein complex (arrows) are indicated.
TFB2/P:\textit{hsp5} complexes, which might occur when the complexes were transferred from the EMSA binding buffer (high salt concentration) to the electrophoresis buffer (low salt concentration). However, the formation of these DNA–protein complexes is obviously due to the specific interaction of TFB2 and the P:\textit{hsp5} DNA but not nonspecific DNA–protein co-aggregation, as such a complex was never generated between TFB2 and the P:\textit{hsp5} mutants in the same EMSA experiments (FM and FD, Figure 7B). Interestingly, although TFBg is also upregulated in \textit{Halobacterium} sp. NRC-1 under heat shock (Table 2), amino acid sequence analysis revealed that TFBb shared more homology with TFB2 than TFBg (TFB2/TFBb, 71%; TFB2/TFBg, 62%). Moreover, microarray data has shown that under low temperature (TFB2/TFBb, 71%; TFB2/TFBg, 62%). Thus, it is likely the expanded family of TFBs plays a much more important role in heat-shock response in these investigated haloarchaea. However, the heat adaptability of TBP in interactions with the TATA box of the heat-shock promoter should not be underestimated. It was observed that the TATA box of P:\textit{hsp5} itself could slightly increase gene expression under heat shock (Figure 5), implying that the corresponding TBP interacts more efficiently with the P:\textit{hsp5} at elevated temperature. This temperature-dependent interaction manner of GTFs with heat-shock promoters was also observed in other archaea, e.g. the TBP and TFB of \textit{Methanosarcina mazeii} were suggested to interact more strongly with stress-gene promoters during heat shock (48). Therefore, it is evident that both TFB and TBP contribute significantly to the upregulation of \textit{hsp5} under heat shock.

It is noteworthy that specific transcriptional repressor modulated heat-shock response has also been reported recently in some thermophilic archaea, such as \textit{P. furiosus} (24,25) and \textit{A. fulgidus} (26); however, these kinds of heat-shock regulators are still not identified in the extremely halophilic archaea. Interestingly, while many haloarchaea encode multiple TBPs and TFBs (19,20,30), some other archaea only harbor one or two TBPs and TFBs. So it is reasonable that haloarchaea have developed an additional sophisticated strategy of gene transcriptional regulation by selection of alternative TFBs and TBPs, as we have revealed in the \textit{hsp5} regulation. This regulatory strategy is conceptually similar to the alternative sigma factors directed transcriptional activation of several heat-shock genes in bacteria (49), and is reminiscent of the HSFs stimulated transcription in eukaryotes (50). Notably, haloarchaea flourish in extremely hypersaline environments and are confronted with many environmental stresses, including frequent changes of temperature.

Transcriptional regulation of the important genes including those for sHSPs by GTFs, but not other secondary regulators, would help haloarchaeal cells respond quickly to the environmental challenges, and thereby adapt more efficiently to the harsh environments.

**ACKNOWLEDGEMENTS**

We would like to thank Mike L. Dyall-Smith for \textit{H. volcanii} DS70, and Dieter Oesterhelt for plasmid pNP22. This work was supported in part by grants from the Ministry of Science & Technology of China (2004CB719603, 2006AA09Z401), the National Natural Science Foundation of China (30621005, 30671141) and Chinese Academy of Sciences (KSCX2-YW-G-023) (to H.X.), and by NSF grants MCB-029617 and 0450695 (to S.D.). Funding to pay the Open Access publication charges for this article was provided by the Chinese Academy of Sciences.

**Conflict of interest statement.** None declared.

**REFERENCES**

1. Allers,T. and Meyarech,M. (2005) Archaea genetics - the third way. \textit{Nat. Rev. Genet.}, 6, 58–73.
2. Reeve,J.N. and Schmitz,R.A. (2005) Biology, biochemistry and the molecular machinery of Archaea. \textit{Curr. Opin. Microbiol.}, 8, 627–629.
3. Sorpa,J. (1999) Transcription initiation in Archaea: facts, factors and future aspects. \textit{Mol. Microbiol.}, 31, 1295–1305.
4. Bell,S.D. and Jackson,S.P. (2001) Mechanism and regulation of transcription in archaea. \textit{Curr. Opin. Microbiol.}, 4, 208–213.
5. Reeve,J.N. (2003) Archaeal chromatin and transcription. \textit{Mol. Microbiol.}, 48, 587–598.
6. Aravind,L. and Koonin,E.V. (1999) DNA-binding proteins and evolution of transcription regulation in the archaea. \textit{Nucleic Acids Res.}, 27, 4658–4670.
7. Bell,S.D., Cairns,S.S., Robson,R.L. and Jackson,S.P. (1999) Transcriptional regulation of an archaeal operon in vivo and in vitro. \textit{Mol. Cell}, 4, 971–982.
8. Brinkman,A.B., Dahlke,I., Tuininga,J.E., Lammers,T., Dumay,V., de Heus,E., Lebbink,J.H., Thomm,M., de Vos,W.M. and van Der Oost,J. (2000) An Lrp-like transcriptional regulator from the archaeon \textit{Pyrococcus furiosus} is negatively autoregulated. \textit{J. Biol. Chem.}, 275, 38160–38169.
9. Bell,S.D. and Jackson,S.P. (2000) Mechanism of autoregulation by an archaeal transcriptional repressor. \textit{J. Biol. Chem.}, 275, 31624–31629.
10. Fiorentino,G., Cannio,R., Rossi,M. and Bartolucci,S. (2003) Transcriptional regulation of the gene encoding an alcohol dehydrogenase in the archaeon \textit{Sulfolobus solfataricus} involves multiple factors and control elements. \textit{J. Bacteriol.}, 185, 3926–3934.
11. Lee,S.J., Engelmann,A., Horflaker,R., Qu,Q., Vierke,G., Hebbeln,C., Thomm,M. and Boos,W. (2003) TrmB, a sugar-specific transcriptional regulator of the trehalose/maltose ABC transporter from the hyperthermophilic archaeon \textit{Thermococcus litoralis}. \textit{J. Biol. Chem.}, 278, 983–990.
12. Krüger,K., Hermann,T., Armbruster,V. and Pfeifer,F. (1998) The transcriptional activator GvpE for the halobacterial gas vesicle genes resembles a basic region leucine-zipper regulatory protein. \textit{J. Mol. Biol.}, 279, 761–771.
13. Pflüger,P. and Pfeifer,F. (2002) A bZIP protein from halophilic archaea: structural features and dimer formation of GvpE from \textit{Halobacterium salinarum}. \textit{Mol. Microbiol.}, 45, 511–520.
14. Ouhammouch,M., Dewhurst,R.E., Hausner,W., Thomm,M. and Geiduschek,E.P. (2003) Activation of archaeal transcription by recruitment of the TATA-binding protein. \textit{Proc. Natl Acad. Sci. USA}, 100, 5097–5102.
15. Bolhuis,H., Palm,P., Wende,A., Falb,M., Ramp,R., Rodriguez-Valera,F., Pfeifer,F. and Oesterhelt,D. (2006) The genome of the square archaeon Haloquadratum walsbyi: life at the limits of water activity. *Genome Res.*, 16, e169.

16. Falb,M., Pfeifer,F., Palm,P., Rodewald,K., Hickmann,V., Tittor,J. and Oesterhelt,D. (2005) Living with two extremes: conclusions from the genome sequence of *Natronomonas pharaonis*. *Genome Res.*, 15, 1336–1347.

17. Baliga,N.S., Bomeau,R., Facciotti,M.T., Pan,M., Glusman,G., Deutsch,E.W., Shannon,P., Chiu,Y., Weng,R.S., Gan,R.R. *et al.* (2004) Genome sequence of *Haloarcula marismortui*: a halophilic archaeon from the Dead Sea. *Genome Res.*, 14, 2221–2234.

18. Ng,W.V., Kennedy,S.P., Mahairas,G.G., Berquist,B., Pan,M., Shiuklal,H., Lasky-S., Baliga,N.S., Thorson,V., Shroga,J. *et al.* (2000) Genome sequence of *Halobacterium* species NRC-1. *Proc. Natl Acad. Sci. USA*, 97, 12176–12181.

19. Baliga,N.S., Goo,Y.A., Ng,W.V., Hood,L., Daniels,C.J. and DasSarma,S. (2000) Is gene expression in *Halobacterium* NRC-1 regulated by multiple TBP and TFI transcription factors? *Mol. Microbiol.*, 36, 1184–1185.

20. Facciotti,M.T., Reess,D.J., Pan,M., Kaur,A., Vuthoori,M., Bomeau,R., Shannon,P., Srivastava.A., Donohoe,S.M., Hood,L.E. *et al.* (2007) General transcription factor specified global gene regulation in archaea. *Proc. Natl Acad. Sci. USA*, 104, 4630–4635.

21. Coker,J.A. and DasSarma,S. (2007) Genetic and transcriptomic analysis of transcription factor genes in the model halophilic Archaeon: coordinate action of TbpD and Tba. *BMC Genet.*, 8, e61.

22. Macario,A.J., Lange,M., Ahring,B.K. and De Macario,E.C. (1999) Stress genes and proteins in the archaea. *Microbiol. Mol. Biol. Rev.*, 63, 923–967.

23. Laksanalamai,P. and Robb,F.T. (2004) Small heat shock proteins from extremophiles: a review. *Extremophiles*, 8, 1–11.

24. Vieker,G., Engelmann,A., Hebbeln,C. and Thomm,M. (2003) A novel archaeal transcriptional regulator of heat shock response. *J. Biol. Chem.*, 278, 18–26.

25. Liu,W., Vierke,G., Wenke,A.K., Thomm,M. and Ladenstein,R. (2005) Heat shock response of *Haloferax volcanii*: a molecular chaperone representing eukaryal and bacterial features. *J. Biol. Chem.*, 280, 189–197.

26. Sun,C., Li,Y., Mei,S., Lu,Q., Zhou,L. and Xiang,H. (2005) A single stress proteins in extreme halophile *Halobacterium* sp. NRC-1: responses to changes in salinity and temperature. *Saline Systems*, 3, e6.

27. Cline,S.W., Lam,W.L., Charlebois,R.L., Schalkwyk,L.C. and Doolittle,W.F. (1989) Transformation methods for halophilic archaea. *Can. J. Microbiol.*, 35, 148–152.

28. Coker,J.A., DasSarma,P., Kumar,J., Müller,J.A. and DasSarma,S. (2007) Transcriptional profiling of the model Archaeon *Haloarcula marismortui* sp. NRC-1: responses to changes in salinity and temperature. *Saline Systems*, 3, e6.

29. Horwitz,J. (1992) Alpha-crystallin can function as a molecular chaperone. *Proteome Sci.*, 3, e6.

30. Yura,T., Nagai,H. and Mori,H. (1993) Regulation of the heat-shock response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev.*, 17, 187–203.

31. Gregor,D. and Pfeifer,F. (2001) Use of a halobacterial *bgaH* reporter gene to analyse the regulation of gene expression in *Haloarcula marismortui*. *Microbiology*, 147, 1745–1754.

32. Merino,R.J. (1999) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev.*, 13, 3788–3796.