Heat-shock sigma factor RpoH from *Geobacter sulfurreducens*

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Recent studies with *Myxococcus xanthus* have suggested that homologues of the *Escherichia coli* heat-shock sigma factor, RpoH, may not be involved in the heat-shock response in this δ -proteobacterium. The genome of another δ -proteobacterium, *Geobacter sulfurreducens*, which is considered to be a representative of the Fe(III)-reducing *Geobacteraceae* that predominate in a diversity of subsurface environments, contains an *rpoH* homologue. Characterization of the *G. sulfurreducens* *rpoH* homologue revealed that it was induced by a temperature shift from 30 °C to 42 °C and that an *rpoH*-deficient mutant was unable to grow at 42 °C. The predicted heat-shock genes, *hrcA*, *grpE*, *dnaK*, *groES* and *htpG*, were heat-shock inducible in an *rpoH*-dependent manner, and comparison of promoter regions of these genes identified the consensus sequences for the –10 and –35 promoter elements. In addition, DNA elements identical to the CIRCE consensus sequence were found in promoters of *rpoH*, *hrcA* and *groES*, suggesting that these genes are regulated by a homologue of the repressor HrcA, which is known to bind the CIRCE element. These results suggest that the *G. sulfurreducens* RpoH homologue is the heat-shock sigma factor and that heat-shock response in *G. sulfurreducens* is regulated positively by RpoH as well as negatively by the HrcA/CIRCE system.

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INTRODUCTION

The Gram-negative δ -proteobacterium *Geobacter sulfurreducens* is considered to be a representative of the Fe(III)-reducing *Geobacteraceae* that predominate in a diversity of subsurface environments where Fe(III) reduction is important (Lovley *et al.*, 2004). *Geobacter* species also play critical roles in bioremediation of groundwater contaminated with organic compounds or metals (Lloyd & Lovley, 2001; Lovley, 1997, 2003; Lovley & Coates, 1997, 2000) and in electricity production from waste organic matter (Bond *et al.*, 2002; Bond & Lovley, 2003; Lovley, 2006a, b). However, the mechanisms they use to deal with the multiple stresses they encounter in these environments are poorly understood.

Geobacter species face various environmental changes and growth conditions in the subsurface. Heat shock is a common stress to which all organisms adapt by inducing heat-shock proteins. Many heat-shock proteins are well conserved among organisms (Arrigo & Iandry, 1994; Lindquist & Craig, 1998). In bacteria, the expression of heat-shock genes is regulated in various fashions (Gross,

1996; Hecker *et al.*, 1996; Narberhaus, 1999; Rosen & Ron, 2002; Schumann, 2000, 2003; Servant & Mazodier, 2001; Yura *et al.*, 2000). The Gram-negative bacterium *Escherichia coli* uses two sigma factors, RpoH and RpoE, to activate transcription of heat-shock genes (Gross, 1996; Yura *et al.*, 2000). The sigma factor, which is a subunit of RNA polymerase (RNAP), recognizes specific promoter elements and is essential for initiation of transcription. RpoH-dependent transcription is also found in other Gram-negative bacteria (Gross, 1996; Yura *et al.*, 2000; Rosen & Ron, 2002). The regulation of heat-shock gene expression in the Gram-positive bacterium *Bacillus subtilis* is rather complex (Hecker *et al.*, 1996; Schumann, 2003). In the *B. subtilis* heat-shock response transcription of heat-shock genes is regulated by both activation via a sigma factor, SigB, and repression via two different transcription factors, HrcA and CtsR. Both HrcA and CtsR repressors are also found in other bacteria (Narberhaus, 1999; Rosen & Ron, 2002). Other repressor proteins, HspR and RheA, have been shown to be involved in heat-shock gene expression in some bacteria (Servant & Mazodier, 2001).

The heat-shock response in δ -Proteobacteria is poorly understood. *Myxococcus xanthus* has three homologues of RpoH (Ueki & Inouye, 2001). However, these homologues were shown to be dispensable for the production of heat-shock proteins and adaptation to heat shock, and instead to be involved in multicellular development in *M. xanthus*

Abbreviations: CIRCE, controlling inverted repeat of chaperone expression; RNAP, RNA polymerase.

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(Ueki & Inouye, 2001). In contrast, *Desulfovibrio vulgaris* has a single homologue of RpoH, and several heat-shock genes were predicted to have RpoH-dependent promoters (Chhabra *et al.*, 2006).

The *G. sulfurreducens* genome contains homologues of the sigma factor genes *rpoD*, *rpoS*, *rpoH*, *rpoE*, *fliA* and *rpoN* (Méthé *et al.*, 2003). In this study, we characterized the role of the *rpoH* homologue in the heat-shock response of the model organism *G. sulfurreducens* in order to understand the stress responses utilized by *Geobacter* communities in the subsurface. Our results indicate that *G. sulfurreducens* RpoH is indeed the heat-shock sigma factor. In addition, it is likely that the HrcA/CIRCE system is also involved in the *G. sulfurreducens* heat-shock response.

METHODS

Bacterial strains and growth conditions. *G. sulfurreducens* DL1 (Caccavo *et al.*, 1994) was used as the parent (wild-type) strain for the construction of the *rpoH* mutant. *G. sulfurreducens* strains were grown anaerobically in NBAF (acetate and fumarate as an electron donor and an acceptor, respectively) medium (Coppi *et al.*, 2001), supplemented with appropriate antibiotics when necessary. *E. coli* DH5 α (Hanahan, 1983) was used for plasmid preparation and grown in LB medium (Miller, 1972), supplemented with appropriate antibiotics when necessary.

Construction of the *rpoH* mutant. The gene encoding RpoH was replaced with a kanamycin-resistance gene, such that the coding region for amino acid residues from Ile-19 to Leu-208 was deleted. Double-crossover homologous recombination was carried out by electroporation (Coppi *et al.*, 2001) with the linear DNA fragment consisting of the kanamycin-resistance gene flanked by 0.7 kb DNA fragments containing the upstream and the downstream regions of *rpoH*. These flanking DNA fragments were amplified by PCR with primer 5'-TCTCTAGATGCCGCCGATGAAAAGATC-3' and 5'-TCGAATTCTCTCAGGTAGACGGTAAGGC-3' (*Xba*I and *Eco*RI sites are underlined), and 5'-TCAAGCTTCCAAGAGTCCGAGT-TGCTC-3' and 5'-TCGGATCCGGACGCACGCGTGGTATC-3' (*Hind*III and *Bam*HI sites are underlined), respectively. The DNA fragment of the kanamycin-resistance gene was amplified by PCR with primers 5'-GCATGAGAATTCTGACGGACACGGGGA-AGTCCAGC-3' and 5'-GCTATGAAGCTTTCATAGAAGGCGGC-GGTGGAATCGAA-3' (*Eco*RI and *Hind*III sites are underlined), and pBBR1MCS-2 (Kovach *et al.*, 1994) as a template. The replacement was confirmed by PCR amplification.

Construction of the expression vector for *rpoH*. The DNA fragment containing the 286 bp upstream region of the initiation codon of RpoH, the coding region of RpoH, and the 102 bp downstream region of the termination codon of RpoH including the putative transcription termination signal was amplified by PCR with primers 5'-TCAAGCTTCTTCAGGACCTCCGTTAGCC-3' and 5'-TCGAATTCATATCGCTCTTGTGATCAC-3' (*Hind*III and *Eco*RI sites are underlined). The spectinomycin-resistance gene was amplified with pJS985Q (Sandler & Clark, 1994) as a template and primers 5'-TCGAATTCACAGGATGACGCCTAAC-3' and 5'-TCCTCGAGTCTAACGCTTGAGTTAA-3' (*Eco*RI and *Xho*I sites are underlined). The *Hind*III–*Eco*RI fragment of the *rpoH* gene and the *Eco*RI–*Xho*I fragment of the spectinomycin-resistance gene were cloned into the *Xho*I–*Hind*III fragment from pCM66 (Marx & Lidstrom, 2001). The resultant plasmid was introduced into the *rpoH* mutant by electroporation (Coppi *et al.*, 2001).

Primer extension assays. *G. sulfurreducens* DL1 (wild-type) and *rpoH* mutant strains were grown at 30 °C before heat shock. Total RNA was prepared before and after heat shock at 42 °C for 10 min. The primers used in the assays were 5'-TTCTCTCAGGTAGACGGTAAGGC-3' (*rpoH*), 5'-TCCTCGATGATGGCTTCGA-3' (*hrcA*), 5'-CCGCGATGGTTTCATCTGCAC-3' (*grpE*), 5'-TCCA-TAACAGCAACGCAGGA-3' (*dnaK*), 5'-CCAGGAGCTGCTGGA-CTTC-3' (*htpG*), and 5'-CGGTCTTGCAACGGTCTGAGATTC-3' (*groES*).

In vitro transcription. The coding region of *G. sulfurreducens* *rpoH* was amplified by PCR with primers 5'-TCTCATATGTCGATGAGCTTACCTGT-3' and 5'-TCGAATTCTCA(GTG)₈GACCGGT-CGCGTCTCTGCAA-3' (*Nde*I and *Eco*RI sites are underlined). The PCR products were cloned into pET24b (Novagen). *G. sulfurreducens* RpoH was prepared as a histidine-tagged protein at the C-terminus (see Fig. 5a). Expression and purification of RpoH were performed as described previously (Ueki & Inouye, 2005). *E. coli* core RNAP and holo RNAP containing RpoD (RNAP/RpoD) were purchased from EPICENTRE Biotechnologies. Holo RNAP/RpoH was reconstituted by mixing *E. coli* core RNAP and *G. sulfurreducens* RpoH and incubating on ice for 15 min before the initiation of transcription reactions. Transcription reactions were conducted at 37 °C. The template contained the *rpoH* promoter region from nucleotides –238 to +42 with respect to the transcription initiation site. *In vitro* transcription reactions were carried out as described by Ueki & Inouye (2002). The transcripts were analysed by a primer extension assay.

RESULTS AND DISCUSSION

G. sulfurreducens RpoH

The *G. sulfurreducens* genome contains a single *rpoH* homologue (GSU0655) (Méthé *et al.*, 2003). *G. sulfurreducens* RpoH shows similarity to RpoH homologues from other bacteria including *G. metallireducens*, *Anaeromyxobacter dehalogenans* and *E. coli*. In addition, *G. sulfurreducens* RpoH contains the sequence QKKLFFKLN, which is highly homologous to the 'RpoH box', a stretch of nine amino acid residues, Q(R/K)(K/R)LFFNLR, that is only conserved in RpoH homologues (Nakahigashi *et al.*, 1995).

To elucidate the function of the *rpoH* homologue in *G. sulfurreducens*, the expression of the *rpoH* gene was examined by a primer extension assay (Fig. 1). *rpoH* mRNA was detected only in cells heat-shocked at 42 °C for 10 min (Fig. 1a). A single 5' end of the mRNA was detected and the putative –35/–10 promoter elements were assigned (Fig. 1b). In addition, a sequence identical to the CIRCE (controlling inverted repeat of chaperon expression) consensus sequence (TTAGCACTC-N₉-GAGTGCTAA), which is the operator sequence bound by HrcA in other bacteria (Narberhaus, 1999; Mogk *et al.*, 1997; Schulz & Schumann, 1996; Zuber & Schumann, 1994), was found to be located in the *rpoH* promoter, indicating that *rpoH* expression is regulated by HrcA as discussed below. It should be noted that a putative transcription termination signal is located downstream of the stop codon (Fig. 1c), indicating that the *rpoH* gene is probably monocistronic.

In order to examine the role of *rpoH* in heat-shock response in *G. sulfurreducens* an *rpoH* mutant was constructed. The mutant grew normally in NBAF medium at 30 °C (data not shown). However, the mutant was unable to adapt to an elevated growth temperature of 42 °C (Fig. 2). The wild-type grew to an OD₆₀₀ of approximately 0.8 after the temperature shift. In contrast, the mutant stopped growing at an OD₆₀₀ of approximately 0.5 after the temperature shift and cell lysis was observed in the mutant culture. The plasmid containing the *rpoH* gene allowed the *rpoH* mutant to grow at 42 °C similarly to the wild-type, indicating that the defect in the adaptation of the *rpoH* mutant was solely due to the absence of the *rpoH* gene. These results suggest that the *rpoH* gene is essential for adaptation to an elevated growth temperature in *G. sulfurreducens*.

Expression of heat-shock genes

To identify genes regulated by RpoH in *G. sulfurreducens*, the expression of genes known to be heat-shock inducible in other bacteria was investigated by primer extension assays (Fig. 3). *G. sulfurreducens* *hrcA* (Fig. 3a), *grpE* (Fig. 3b), *dnaK* (Fig. 3c), *htpG* (Fig. 3d), and *groES* (Fig. 3e) were induced by heat shock at 42 °C for 10 min. One 5' end of *htpG* mRNA was detected, two 5' ends of mRNA were detected for *hrcA*, *grpE* and *dnaK*, and three 5' ends of *groES* mRNA were detected. The expression of these genes was dependent on the *rpoH* gene, as it was undetectable or drastically decreased in the *rpoH* mutant. Interestingly, *groES* mRNA was observed even before heat shock, although the expression level was low.

This suggests that *groES* and most likely *groEL*, which is located downstream of *groES* and appears to be co-transcribed with *groES*, are required at physiological temperatures. Molecular chaperonins such as the GroE system and molecular chaperones such as the DnaK/DnaJ/GrpE and the HtpG systems are crucial to protein folding (Riggs *et al.*, 2004; Young *et al.*, 2004; Zhang *et al.*, 2002). In addition, the GroE system is thought to activate the HrcA repressor (Mogk *et al.*, 1997; Schumann, 2000).

The putative $-35/-10$ promoter elements were assigned for these genes from an analysis of the 5' ends identified by the primer extension assays (Fig. 3). A sequence identical to the CIRCE consensus sequence (Narberhaus, 1999) was found to be located in the *hrcA* promoter, indicating that HrcA represses the transcription of its own gene. Such auto-regulation of *hrcA* is also found in other bacteria, such as the ϵ -proteobacterium *Helicobacter pylori* (Spohn *et al.*, 2004). In addition, the *groES* promoter contains a sequence identical to the CIRCE consensus sequence, as other *groES* promoters do in bacteria that utilize HrcA. The $-35/-10$ elements in the *hrcA* P2, the *grpE* P2, the *dnaK* P2, the *groES* P3 and the *htpG* promoters show high similarity to one another and those in the *rpoH* promoter (Fig. 4a). The *groES* P1 promoter has $-35/-10$ elements similar to those in the RpoD-dependent promoters, while the other promoters exhibit no apparent similarity to known promoters. These results indicate that these heat-shock genes and *rpoH* are regulated by the *rpoH* gene. Moreover, it is likely that the absence and/or the reduced expression of heat-shock genes

Fig. 1. Expression of the *rpoH* gene. (a) Primer extension assay. Total RNA was prepared before (–) and after (+) heat shock. (b) The promoter region of the *rpoH* gene. The 5' end of the *rpoH* mRNA identified in (a) is indicated by +1. The putative -35 and -10 elements and putative ribosome-binding site (RBS) are underlined. The arrows indicate the putative CIRCE element. The initiation codon is indicated by Met. (c) The transcription termination of the *rpoH* gene. The arrows indicate the putative transcription termination signal. The stop codon is underlined.

Fig. 2. Adaptation to a higher temperature. The wild-type (circles), the *rpoH* mutant (squares), and the *rpoH* mutant harbouring the plasmid containing the *rpoH* gene (triangles) were grown at 30 °C and then shifted to 42 °C. Growth was monitored as OD₆₀₀.

caused by the lack of the *rpoH* gene results in inadequate adaptation to the higher temperature.

The consensus sequences for the $-35/-10$ promoter elements of heat-shock genes in *G. sulfurreducens* are proposed (Fig. 4a). The -35 element shows high similarity to that of the *E. coli* RpoH consensus recognition sequence (Gross, 1996; Yura *et al.*, 2000), whereas the -10 element exhibits low similarity. This difference is supported by the extent of similarity in amino acid sequences of RpoH from *G. sulfurreducens* and *E. coli* (Fig. 4b). Region 4.2, which recognizes the -35 element, is highly similar between *G. sulfurreducens* and *E. coli* RpoH homologues, while region 2.4, which recognizes the -10 element, is less conserved between *G. sulfurreducens* and *E. coli* RpoH homologues.

The gene cluster containing *hrcA*, *grpE*, *dnaK* and *dnaJ*

In *G. sulfurreducens*, *hrcA*, *grpE*, *dnaK* and *dnaJ* are located in this order on the chromosome and appear to constitute an operon, because the lengths of intergenic regions between these genes are short (data not shown). A similar gene organization of these homologues is also found in other bacteria such as *B. subtilis* (Wetzstein *et al.*, 1992). *grpE* is typically located downstream of *hrcA* on the chromosome in bacteria that contain *hrcA*. Despite the proximity between *hrcA* and *grpE*, the transcription of *grpE* is often driven by its own promoter (Wetzstein *et al.*, 1992; Narberhaus *et al.*, 1992; Roberts *et al.*, 1996). In contrast, the location of *dnaK* and *dnaJ*, which is usually located downstream of *dnaK*, is diversified. When located downstream of *grpE*, *dnaK* and *dnaJ* are co-transcribed with *grpE* (Wetzstein *et al.*, 1992; Narberhaus *et al.*, 1992). However, in *G. sulfurreducens*, not only *grpE* but also *dnaK* were individually transcribed (Fig. 3). It appears likely that *dnaJ* is co-transcribed with *dnaK* in *G. sulfurreducens*, as a 5' end specific to *dnaJ* mRNA

was not detected by a primer extension assay (data not shown). Separate transcription may facilitate more efficient expression of these genes than co-transcription, resulting in proper function of these genes during heat shock. However, it is possible that these genes can be both co-transcribed and individually transcribed under different conditions.

Transcriptional regulation of *rpoH*

To investigate the transcriptional regulation of the *rpoH* gene in *G. sulfurreducens*, *in vitro* transcription assays were conducted with the *rpoH* promoter as a template (Fig. 5). The *in vitro* transcripts were analysed by primer extension assay to confirm that the 5' end of the *in vitro* transcripts was the same as that of the *in vivo* transcripts. Holo RNAP/RpoH recognized the *rpoH* promoter and initiated transcription *in vitro* from the same position as *in vivo* (Fig. 5b), indicating that RNAP/RpoH transcribes its own gene. Furthermore, it is likely that RNAP/RpoH initiates the transcription of the other heat-shock genes, as their expression was dependent on *rpoH* and their promoter elements show high similarity to *rpoH* promoter elements (Fig. 4a). Surprisingly, *E. coli* holo RNAP/RpoD also initiated transcription *in vitro* from the same position as *in vivo*, although it produced fewer transcripts than RNAP/RpoH. The regulation of *rpoH* expression *in vivo* was further examined in the *rpoH* mutant (Fig. 5c). It was found that *rpoH* expression was still induced by heat shock in the *rpoH* mutant, although its expression level decreased. It should be noted that the *rpoH* promoter region as well as the 5' end of the coding region, to which the primer used in the primer extension assays hybridized, were not deleted in the *rpoH* mutant, allowing one to measure the *rpoH* promoter activity in the *rpoH* mutant. These results suggest that the *rpoH* promoter is also recognized by RNAP/RpoD and that RNAP/RpoD is capable of transcribing the *rpoH* gene in the absence of RpoH. *rpoD* is known to be heat-shock inducible in some bacteria such as *E. coli* (Taylor *et al.*, 1984). Thus, it is possible that *G. sulfurreducens* *rpoD* is also heat-shock inducible and that the induction of RpoD is sufficient to account for transcription of *rpoH* in the *rpoH* mutant. The *rpoH* promoter region contains the sequences TTGATT and TACATT (Fig. 1b), which show similarity to the *E. coli* RpoD consensus sequences TTGACA and TATAAT, respectively (Harley & Reynolds, 1987; Hawley & McClure, 1983). In addition, the *groES* P1 promoter also contains the RpoD recognition sequence-like $-35/-10$ elements, TTGATT and TATAGT, respectively, and *groES* expression dependent on the P1 promoter was still induced by heat shock in the *rpoH* mutant (Fig. 3e). These results further suggest that RpoD is involved in heat-shock response in *G. sulfurreducens*. However, it is also possible that instead of RpoD, RpoS is involved in their expression in *G. sulfurreducens*. RpoS is the stationary phase sigma factor involved in responses to various stresses including heat shock (Hengge-Aronis, 2002) and *G. sulfurreducens* possesses a homologue of RpoS (Núñez *et al.*, 2004). Furthermore, RpoD and RpoS recognize similar $-35/-10$ promoter elements (Yan *et al.*, 2006).

The expression of RpoH is mainly controlled at the level of translation in the Gram-negative γ -proteobacterium *E. coli* (Gross, 1996; Yura *et al.*, 2000). Upon heat shock the cellular level of RpoH increases by both enhanced translation of *rpoH* mRNA and stabilization of RpoH in *E. coli*. The *rpoH* homologues from γ -Proteobacteria share common structural characteristics with *E. coli rpoH*, such as a downstream box, mRNA secondary structure and highly conserved amino acid sequence of region C, all of which are important for thermoregulation of *rpoH* translation and for stability and activity of RpoH in *E. coli* (Nakahigashi *et al.*, 1995). In

contrast, α - and β -Proteobacteria have diverged from γ -Proteobacteria in their mechanisms of regulation of *rpoH* expression. *rpoH* genes from α - and β -Proteobacteria do not contain characteristics found in those from γ -Proteobacteria. Instead, some *rpoH* genes from α -Proteobacteria, such as *Agrobacterium tumefaciens* (Nakahigashi *et al.*, 1999), *Bradyrhizobium japonicum* (Narberhaus *et al.*, 1997) and *Caulobacter crescentus* (Reisenauer *et al.*, 1996; Wu & Newton, 1996), contain an RpoH-dependent promoter that can be induced by heat shock. In *G. sulfurreducens* the downstream box and mRNA secondary structure found in

Fig. 3 (continued from facing page). Expression of heat-shock genes. (a) *hrcA*. (b) *grpE*. (c) *dnaK*. (d) *htpG*. (e) *groES*. Primer extension assays and their promoter regions are shown. Total RNA was prepared from the wild-type (WT) and the *rpoH* mutant ($\Delta rpoH$) strains before (–) and after (+) heat shock. The 5' ends of mRNA identified by the primer extension assays are indicated by +1. The putative –35 and –10 elements and putative RBS are underlined. The arrows indicate the putative CIRCE element. The initiation codon is indicated by Met.

γ -Proteobacteria are absent (data not shown), while the *rpoH* gene has an RpoH-dependent promoter (Figs 1 and 4). Thus, the regulation of *rpoH* expression in *G. sulfurreducens* appears to be more closely related to that in α -Proteobacteria. However, it is likely that *rpoH* expression in *G. sulfurreducens* is more tightly regulated, as *G. sulfurreducens* *rpoH* also contains a DNA element identical to the CIRCE consensus sequence (Fig. 1), indicating negative regulation of *rpoH* expression by HrcA.

Heat-shock promoters in *G. sulfurreducens*

The heat-shock promoters in *G. sulfurreducens* can be classified into four groups: promoters containing –35/–10

elements similar to the RpoH consensus recognition sequences (group 1; *grpE* P2, *dnaK* P2 and *htpG*), ones containing both –35/–10 elements similar to the RpoH consensus recognition sequences and the sequence identical to the CIRCE consensus sequence (group 2; *rpoH*, *hrcA* P2 and *groES* P3), ones containing –35/–10 elements similar to the RpoD consensus recognition sequences (group 3; *groES* P1), and ones containing –35/–10 elements different from the consensus recognition sequences for RpoH, RpoD or RpoS (group 4; *hrcA* P1, *grpE* P1, *dnaK* P1 and *groES* P2). The expression of group 1 during heat-shock response was undetectable in the *rpoH* mutant, while the expression dependent on the promoters containing the CIRCE

Fig. 4. Heat-shock promoters. (a) Comparison of *G. sulfurreducens* heat-shock promoters and *E. coli* RpoH consensus recognition sequences (Gross, 1996; Yura *et al.*, 2000). Nucleotides conserved in more than two promoters are highlighted. (b) Comparison of regions 4.2 and 2.4 of RpoH from *G. sulfurreducens* and *E. coli*. Identical amino acid residues are highlighted.

sequence was still observed (Figs 3 and 5), suggesting that the expression of *rpoH*, *hrcA* and *groES* is repressed by the HrcA/CIRCE system. The presence of the -35/-10 elements similar to those in RpoD- or RpoS-dependent promoters suggests the involvement of RpoD and/or RpoS in heat shock. Because there is no apparent sequence similarity among -35/-10 promoter elements in group 4, it

is possible that another transcription factor is involved in heat-shock response transcription.

Phylogenetic perspectives

The division of *δ-Proteobacteria* consists of a variety of Gram-negative bacteria including anaerobic metal-reducing

Fig. 5. Transcription of *rpoH*. (a) Purification of *G. sulfurreducens* RpoH. The sizes of molecular standards are shown in kDa. (b) *In vitro* transcription. The *rpoH* promoter was tested as a template with core RNAP, holo RNAP/RpoD and RNAP/RpoH. Transcripts were analysed by primer extension assays with the same primer as used in Fig. 1(a). *In vivo* RNA was used as a control for the transcription initiation site. (c) *In vivo* expression. Total RNA was prepared from wild-type (WT) and *rpoH* mutant (Δ rpoH) strains before (-) and after (+) heat shock and analysed by primer extension assays with the same primer as used in Fig. 1(a).

bacteria such as *Geobacter* and *Desulfovibrio* species, bacteriolytic *Bdellovibrio* species, syntrophic bacteria and aerobic developmental myxobacteria. RpoH homologues were identified in other δ -*Proteobacteria* including *G. metallireducens* (CP000148), *D. vulgaris* (AE017285), *Bdellovibrio bacteriovorus* (BX842601), the syntrophic benzoate-oxidizing bacterium *Syntrophus aciditrophicus* (CP000252), *M. xanthus* (CP000113), and an anaerobic myxobacterium, *A. dehalogenans* (CP000251) (www.ncbi.nlm.nih.gov/Genomes). Most of the δ -*Proteobacteria* described above have a single RpoH homologue, while *M. xanthus* and *A. dehalogenans* have three RpoH homologues. In addition, RpoH-dependent promoters were predicted to be present in several genes in anaerobic metal-reducing δ -*Proteobacteria* (Rodionov *et al.*, 2004). Thus, it appears likely that most of the δ -*Proteobacteria* have RpoH-dependent heat-shock response transcription as found in *G. sulfurreducens*.

HrcA homologues were identified in other δ -*Proteobacteria* including *G. metallireducens*, *D. vulgaris*, *M. xanthus* and *A. dehalogenans*, whereas they are absent from *B. bacteriovorus* and *S. aciditrophicus* (www.ncbi.nlm.nih.gov/Genomes). In addition, the CIRCE consensus sequence was predicted to be located in several genes from anaerobic metal-reducing δ -*Proteobacteria* (Rodionov *et al.*, 2004). Thus, the HrcA/CIRCE negative regulatory system may also be involved in transcription of heat-shock genes in δ -*Proteobacteria*.

Based on the genomic analyses described above, it appears likely that transcription mechanisms during heat-shock response are diverse in δ -*Proteobacteria*. Anaerobic metal-reducing bacteria utilize both RpoH as an activator and HrcA as a repressor for transcription of heat-shock genes. A developmental myxobacterium, *M. xanthus*, seems to contain only the HrcA/CIRCE system. Bacteriolytic and syntrophic microorganisms, *B. bacteriovorus* and *S. aciditrophicus*, respectively, appear to possess only RpoH-dependent transcription.

Apparent homologues of other repressors known to be involved in bacterial heat-shock response transcription such as CtsR, HspR and RheA are absent from *G. sulfurreducens*. However, it is possible that a regulatory system unidentified in other bacteria is present in *G. sulfurreducens* as well as other δ -*Proteobacteria*. For instance, *M. xanthus* utilizes the HsfAB two-component system to activate *lonD* expression upon heat shock (Ueki & Inouye, 2002).

Conclusions

The results demonstrate that the heat-shock sigma factor, RpoH, is essential for adaptation to a higher temperature in *G. sulfurreducens*. Furthermore, it is most likely that the HrcA/CIRCE repression system is also involved in heat-shock response transcription in *G. sulfurreducens*. Taken together with the genomic information, the mechanisms of heat-shock response transcription appear to be diversified in the Gram-negative δ -*Proteobacteria*. Temperature is one of

important environmental factors that influence microbial activities in the subsurface. This study will serve as a foundation for further characterization of *Geobacter* species in adaptation to different temperatures, which should allow optimization of conditions for applications of *Geobacter* species to bioremediation and electricity production.

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