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Translational control of small heat shock genes in mesophilic and thermophilic cyanobacteria by RNA thermometers

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Supplementary Information

Fig. S1 Sequence alignment of *hspA*-5´UTR from *Thermosynechococci*.

Table S1 Strains, plasmids, and oligonucleotides used in this study

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Fig. S1

Fig. S1 Sequence alignment of *hspA* upstream region from *Thermosynechococci*.
The sequence alignment was performed using ClustalW2 [1, 2], asterisks mark similar positions. Promoter sequence, transcriptional start site (*), Shine-Dalgarno sequence (SD), and ATG are depicted in bold. The *hspA* nucleotide sequence from *T. vulcanus* was retrieved from [3], as well as the highlighted inverted repeat, a putative DNA protein binding site. The transcriptional start site was mapped before. [4] The *hspA* upstream region from both *Thermosynechococci* is highly similar, the 5´UTR just being elongated by 6 nt. Therefore, the same transcriptional start site as for *T. vulcanus hspA* was assumed for *T. elongatus hspA*.

Strain, plasmid, or oligonucleotide	Relevant characteristic or Sequence	Source or Reference
Strains		
Escherichia coli DH5α	*supE44*, Δ*lacU169* (Φ80d*lacZ*ΔM15), *hsdR17*, *recA1*, *endA1*, *gyrA96*, *thi-1*, *relA1*	[5, 6] [7] [8] [9]
Thermosynechococcus elongatus BP-1	Wild type	
Anabaena variabilis ATCC 29413	Wild type	
Nostoc sp. PCC 7120 (*Anabaena* sp. PCC 7120)	Wild type	
Plasmids		
pUC18	Cloning vector, Ap^r	[10]
pBAD2-*bgaB*	Arabinose inducible; reporter gene system for translational fusions to *bgaB*; Ap^r	[11]
pBAD2-*hsp17* (pBO1836)	pBAD2-*bgaB* based, *Synechocystis hsp17*-5´UTR plus 2 codons fused to *bgaB*	[11]
pT7*hspA*-cdr (pBO1858)	pUC18 based, contains T7 promoter, *hspA*-5´UTR plus 20 codons, HpyCH4v	This study
pUC18-*avashort* (pBO2154)	pUC18 cloning vector harboring *ava3076*-5´UTR plus 2 codons (NheI, EcoRI)	This study
pUC18-*avalong* (pBO2155)	pUC18 cloning vector harboring *ava4812*-5´UTR plus 2 codons (NheI, EcoRI)	This study
pUC18-*alrshort* (pBO2761)	pUC18 cloning vector harboring *alr0286*-5´UTR plus 2 codons (NheI, EcoRI)	This study
pUC18-*alrlong* (pBO2762)	pUC18 cloning vector harboring *alr1809*-5´UTR plus 2 codons (NheI, EcoRI)	This study
pBAD2-*avashort* (pBO2161)	pBAD2-*bgaB* based, insert cloned from pUC18-*avashort* via NheI, EcoRI	This study
pBAD2-*avalong* (pBO2162)	pBAD2-*bgaB* based, insert cloned from pUC18-*avalong* via NheI, EcoRI	This study
pBAD2-*alrshort* (pBO2795)	pBAD2-*bgaB* based, insert cloned from pUC18-*alrshort* via NheI, EcoRI	This study
pBAD2-*alrlong* (pBO2796)	pBAD2-*bgaB* based, insert cloned from pUC18-*alrlong* via NheI, EcoRI	This study
pT7*avalong* (pBO2772)	pUC18 based, contains T7 promoter, *avalong*-5´UTR plus ATG, EcoRV	This study
pT7*avashort*-cdr (pBO2775)	pUC18 based, contains T7 promoter, *avashort*-5´UTR plus 24 codons, EcoRV	This study
pBAD2-*avalong*Δloop1 (pBO2799)	pBAD2-*avalong*-based, site directed mutagenesis with primers 712, 713	This study
pBAD2-*avalong*ΔT90 (pBO3051)	pBAD2-*avalong*-based, site directed mutagenesis with primers 716, 717	This study
pBAD2-*avalong*CC88/91AA (pBO2816)	pBAD2-*avalong*-based, site directed mutagenesis with primers 1278, 1279	This study
pBAD2-*avalong*2A (pBO2793)	pBAD2-*avalong*-based, site directed mutagenesis with primers 980, 981	This study

pBAD2-*avalong*4A (pBO2794)	pBAD2-*avalong*-based, site directed mutagenesis with primers 1003, 1004	This study
pBAD2-*avashort*-antiSD1 (pBO2751)	pBAD2-*avashort*-based, randomized substitution of C34, C35 by T34, A35	This study
pBAD2-*avashort*-antiSD2 (pBO2752)	pBAD2-*avashort*-based, randomized substitution of C34, C35 by T34, T35	This study
pBAD2-*avashort*-antiSD3 (pBO2753)	pBAD2-*avashort*-based, randomized substitution of C34, C35 by G34, A35	This study
pBAD2-*avashort*-antiSD4 (pBO2754)	pBAD2-*avashort*-based, randomized substitution of C34, C35 by G34, G35	This study
pBAD2-*avashort*C46A (pBO2798)	pBAD2-*avashort*-based, site directed mutagenesis with primers 734, 735	This study

Oligonucleotides

371 T7*hspA*for	*GAAATTAATACGACTCACTATAGGG*GGGTAGAGGGAATCAGCCCAAG (pBO1858)
372 *hspA*_cdrev	TGCAGTTCATTTGGCGTTGAATTGC (pBO1858)
552 for-*bgaB*3076	TTGCTAGCAAGATTAAAGCCTGGAAGCG (pBO2154, RNA probe forward primer))
553 rev-*bgaB*3076	TTGAATTCTGTCATATCTTTGACTCCTC (pBO2154)
550 for-*bgaB*4812	TTGCTAGCGAAGGAGCTAGAGATTAAGC (pBO2155, RNA probe forward primer)
551 rev-*bgaB*4812	TTGAATTCTGCCATAATATCACCTCTAA (pBO2155, pBO2762)
1002 for-*bgaB*0286	TTGCTAGCAAATGAAAGATTAAAGCCTG (pBO2761)
668 Revalr0286	TTGAATTCTGTCATAGTTTTGACTCCTC (pBO2761)
526 for-*bgaB*1809	TTGCTAGCGAAGGAGCTAGAAATTAAGC (pBO2762)
862 T73076for	TT*GAAATTAATACGACTCACTATAGG*AAGATTAAAGCCTGGAAGCG (pBO2775)
857 3076_72cdrev	TTGATATCCTCAAACAAATGATTTAA (pBO2775)
859 T74812for	TT*GAAATTAATACGACTCACTATAGG*GAAGGAGCTAGAGATTAAGC (pBO2772)
860 4812bluntrev	TTGATATCCATAATATCACCTCTAAAATGC (pBO2772)
712 4812dloop_for	GATTAAGCTTTTCCACAAAAAAGCTTAATAATAGC (pBO2799)
713 4812dloop_rev	GCTATTATTAAGCTTTTTTGTGGAAAAGCTTAATC (pBO2799)
716 4812dT90_for	GCTTAATAATAGCCTCTGATAAGCAAATGTTG (pBO3051)
717 4812dT90_rev	CAACATTTGCTTATCAGAGGCTATTATTAAGC (pBO3051)
1278 QCC88/91Afw	GCTTAATAATAGCATTATGATAAGCAAATGTTGC (pBO2816)
1279 QCC88/91Arv	GCAACATTTGCTTATCATAATGCTATTATTAAGC (pBO2816)
710 3076QC_for	GCGATTCGCAGGAAAA**DD**TTAGAGGAGTCAAAG (pBO2751-2754)
711 3076QC_rev	CTTTGACTCCTCTAA**HH**TTTTCCTGCGAATCGC (pBO2751-2754)
734 3076_C46A_for	GGAAAACCTTAGAGGAGTAAAAGATATGACAGAATTC (pBO2798)
735 3076_C46A_rev	GAATTCTGTCATATCTTTTACTCCTCTAAGGTTTTCC (pBO2798)

529 RACE_3076	TCTTGCCAAGGGTTCCAACG
461 RACE_4812	TCTTCAAGTTCAGCAGCAGG
Adapter for 5´RACE	GTCAGCAATCCCTAAC*GAG (GAG ribonucleotides)*
Adapter primer for 5´RACE	GTCAGCAATCCCTAACGAG
1028 avashort_RNAprobe_rev	*GAAATTAATACGACTCACTATAGGG*AGGTAATGTCAAATTCAAG
1029 avalong_RNAprobe_rev	*GAAATTAATACGACTCACTATAGGG*AGCCAGCAGCAGATTCAATG
980 Qc_c53-4af	CAAATTAATTCAACAATATAAATCTTATTTAATTAGTAAAAAGC (pBO2793)
981 Qc_c53-4ar	GCTTTTTACTAATTAAATAAGATTTATATTGTTGAATTAATTTG (pBO2793)
1003 QCf4812_53-6A	AAATTAATTCAACAATATAAAAATTATTTAATTAGTAAAAAG (pBO2794)
1004 QCr4812_53-6A	CTTTTTACTAATTAAATAATTTTTATATTGTTGAATTAATTT (pBO2794)

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