

Supplementary Data

Structural basis of transcriptional regulation by the HigA antitoxin

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Data deposition: Crystallography, atomic coordinates, and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB codes 6CF1, 6CHV)

Key words: toxin-antitoxin complex, DNA-binding protein, stringent response, transcriptional repressor

Supplementary Tables

Table S1. Crystallographic statistics.

	HigA	HigA-higO2
Data Collection		
Space group	C2	P6 ₅
Cell dimensions	105 40.7 45.3	79.4 79.4 399
a, b, c,	90 105.5 90	90 90 120
α , β , γ ,		
Resolution (Å)	44 - 1.9 (2.0 - 1.9)	41 - 2.9 (3.0 - 2.9)
R _{merge} (%)	11 (69)	13 (42)
R _{pim} (%)	4.8 (29)	3.6 (12)
CC1/2 (%)	99 (83)	99 (99)
I/ σ I	11 (2.5)	12 (3.2)
Completeness (%)	99 (99)	100 (100)
Redundancy	6.5 (6.5)	14 (14)
Refinement		
Resolution	44 - 1.9	41 - 2.9
No. unique reflections	14530	31822
R _{work} /R _{free} (%)	18.0/21.8	22.5/26.0
R.m.s. deviations		
Bond lengths	0.014	0.004
Bond angles	1.45	0.59

Table S2. Strains.

Strain	Genotype
BW25113	<i>Escherichia coli</i> Δ (araBAD)567 Δ (rhaBAD)568 Δ lacZ4787(::rrnB-3) hsdR514 rph-1
BL21(DE3)	<i>Escherichia coli</i> strain B F ⁻ ompT gal dcm lon hsdS _B (r _B ⁻ m _B ⁻) λ (DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) [malB ⁺] _{K-12} (λ^S)
ECJM901	BL21(DE3) pET28a-his6-Rts1 higA
ECJM730	BW25113 pBAD33
ECJM765	BW25113 pBAD33-Rts1 higB
ECJM821	BW25113 pBAD33-Rts1 higB-higA
ECJM898	BW25113 pBAD33-Rts1 higB-higA Δ 84-104
ECJM820	BW25113 pQF50-Rts1 Phig-lacZ
ECJM908	BW25113 pQF50-Rts1 Phig-higA-lacZ
ECJM909	BW25113 pQF50-Rts1 Phig- higA Δ 84-104-lacZ
ECJM833	BW25113 pQF50-Rts1 Phig-lacZ pBAD33
ECJM885	BW25113 pQF50-Rts1 Phig-lacZ pBAD33-Rts1 higA
ECJM900	BW25113 pQF50-Rts1 Phig-lacZ pBAD33-Rts1 higA Δ 84-104
ECJM925	BW25113 pBAD33-Rts1 higB-higA R40A
ECJM914	BW25113 pQF50-Rts1 Phig-lacZ pBAD33-Rts1 higA R40A
ECJM902	BL21(DE3) pET28a-his6-Rts1 higA R40A

Table S3. Plasmids.

Plasmid	Description or reference
pBAD33	(Guzman et al. 1995)
pQF50	(Farinha and Kropinski 1990)
pET28a-his6-Rts1 higA	(Hurley and Woychik 2009)
pJM346	pBAD33-Rts1 <i>higB</i>
pJM362	pBAD33-Rts1 <i>higB-higA</i>
pJM380	pBAD33-Rts1 <i>higB-higAΔ84-104</i>
pJM359	pQF50-Rts1 <i>Phig-lacZ</i>
pJM384	pQF50-Rts1 <i>Phig-higA-lacZ</i>
pJM385	pQF50-Rts1 <i>Phig-higAΔ84-104-lacZ</i>
pJM360	pBAD33-Rts1 <i>higA</i>
pJM378	pBAD33-Rts1 <i>higAΔ84-104</i>
pJM381	pBAD33-Rts1 <i>higB-higA R40A</i>
pJM386	pBAD33-Rts1 <i>higA R40A</i>
pJM382	pET28a-his6-Rts1 <i>higA R40A</i>

Table S4. Oligonucleotides.

Oligo	Sequence
oJM821	cgctgcagacaccatgtaatacagagatgg
oRJD2	aataataagcttagtgataatcctcataatttaaaatgtaagcatcacc g
oJM837	cgcaagcttttaatgctcttgcgcgttctg
oJM862	cgcaagcttttagatttggttttctaactggcgag
oJM835	ggtattgcacatcgtgtaatatcgcggtatagtattacacaccatgtaa taca
oJM836	agcttgtattacatgggtgtgtaatactataaccgcatattacacgatgt gcaatacctgca
oJM863	cgtattgcacatcgtgtaatatcgcggtatagtattacacaccatgtaa tacg
oJM864	gatccgtattacatgggtgtgtaatactataaccgcatattacacgatgt gcaatacgcgatg
oJM865	cgcgatccttttaaattatgaggattatcactaatg
oJM823	cgctgcagtagagggtattaataatgagacaattcaaggtttcac
pHigA_F	tgtattgcacatcgtgtaatatcgcggtatagtattacacaccatgtaa tacagagatggc
pHigA_R	gccatctctgtattacatgggtgtgtaatactataaccgcatattacacg atgtgcaataca
pHigA_F_Scra1	tcagccatgtggacacccccggcgcggtatagtattacacaccatgtaa tacagagatggc
pHigA_R_Scra1	gccatctctgtattacatgggtgtgtaatactataaccgcgccgggggtgt ccacatggctga
pHigA_F_Scra2	tgtattgcacatcgtgtaatatcgcggtatacagccatgtggacacccc cggagagatggc

pHigA_R_Scra2	gccatctctccgggggtgtccacatggctgtataccgcgatattacacg atgtgcaataca
pHigCryst3	gtattacacaccatgtaatac
pHigCryst4	gtattacatgggtgtgtaatac
oJM870	cgtatccgctctacttgcggggaaaactgcggttgaccct
oJM871	gcaagtagagcggatacggttgctggtgtaacaccaatattgtgagc
oJM868	tcagccatgtggacacccccggcgcggtatagtattatacaccatataa tacagagatggc
oJM869	gccatctctgtattatatgggtgtataataactataccgcgccgggggtgt ccacatggctga

Supplementary Figures

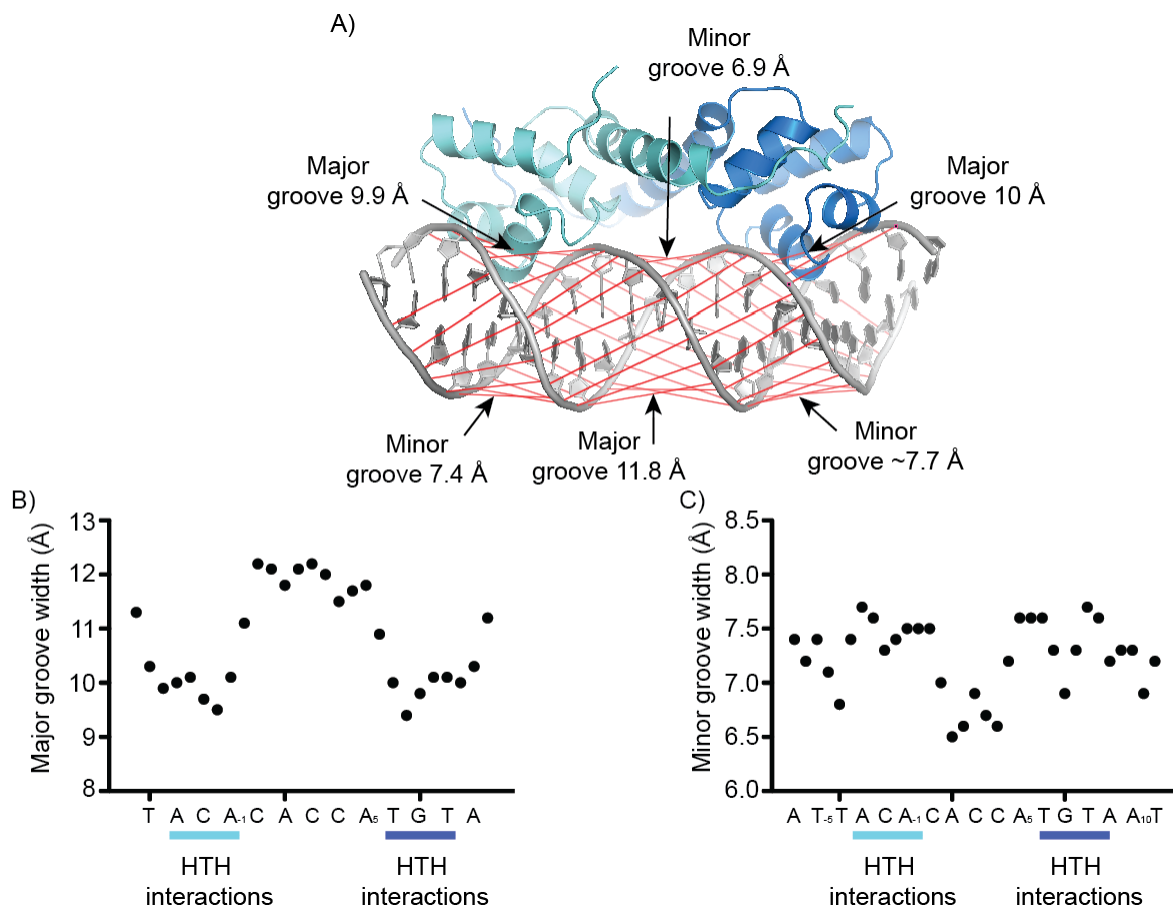


Figure S1. Analysis of *higO2* major and minor groove widths. A) Structure of HigA bound to *higO2* with major and minor groove distances calculated by Curves+ depicted as red lines. B) Major groove and C) minor groove distances calculated by Curves+. The van der Waals surface of the phosphodiester backbone was kept at 2.9 Å when calculating the major and minor groove widths.

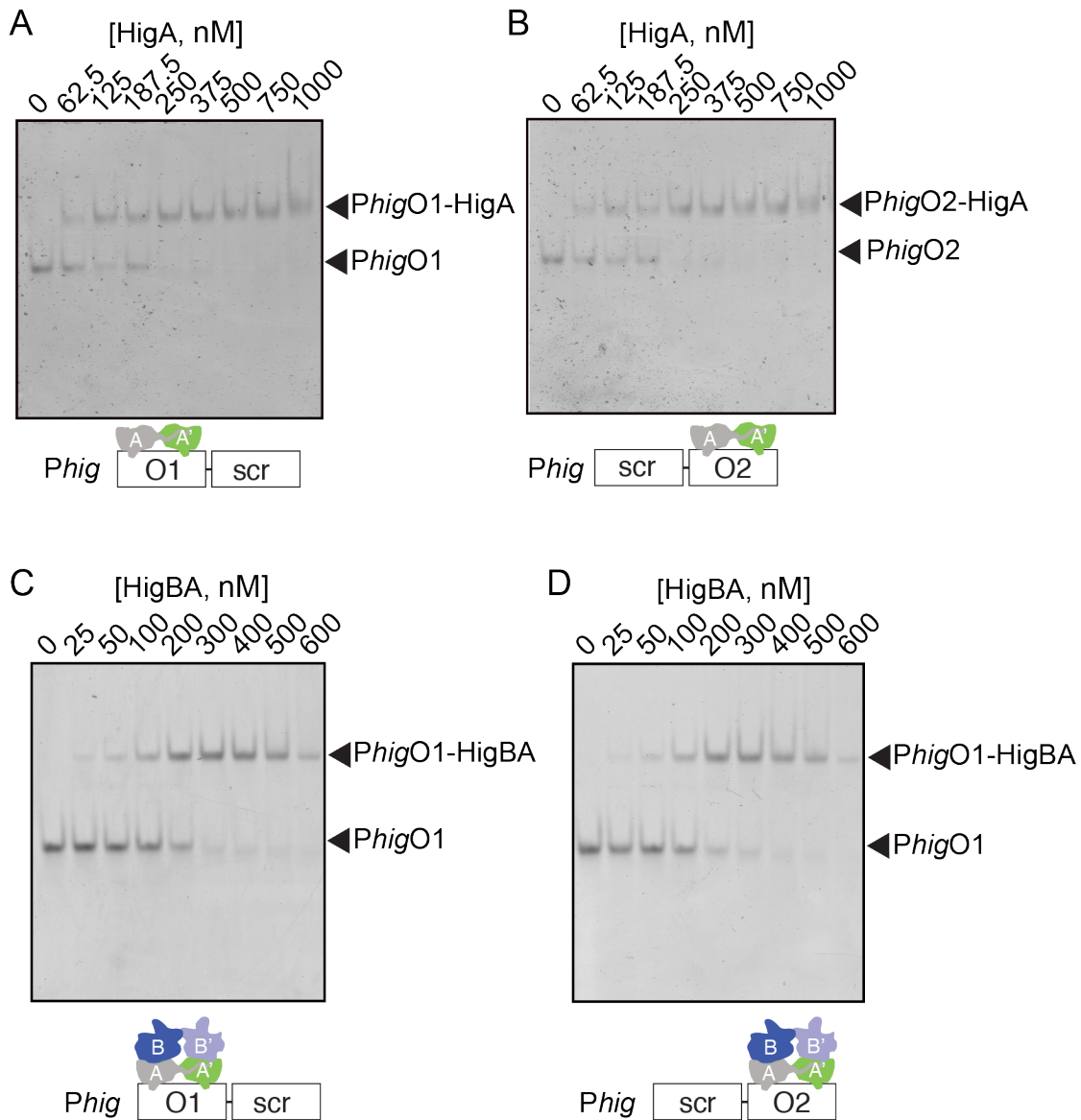


Figure S2. HigA and HigBA recognition of *higO1* or *higO2*. Electrophoretic mobility shift assays of HigA protein and *Phig* containing *higO1* and a scrambled *higO2* (A), HigA protein and *Phig* containing *higO2* and a scrambled *higO1* (B), HigBA protein and *Phig* containing *higO1* and a scrambled *higO2* (C), and HigBA protein and *Phig* containing *higO2* and a scrambled *higO1*. Under each panel is a schematic of the interactions of the HigA dimer and the HigBA complex with *PhigO1*-*O2*. Each HigA and HigB monomer are labeled as A and A', and B and B', respectively. HigA and HigBA concentrations are shown in nM above each gel.

References

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