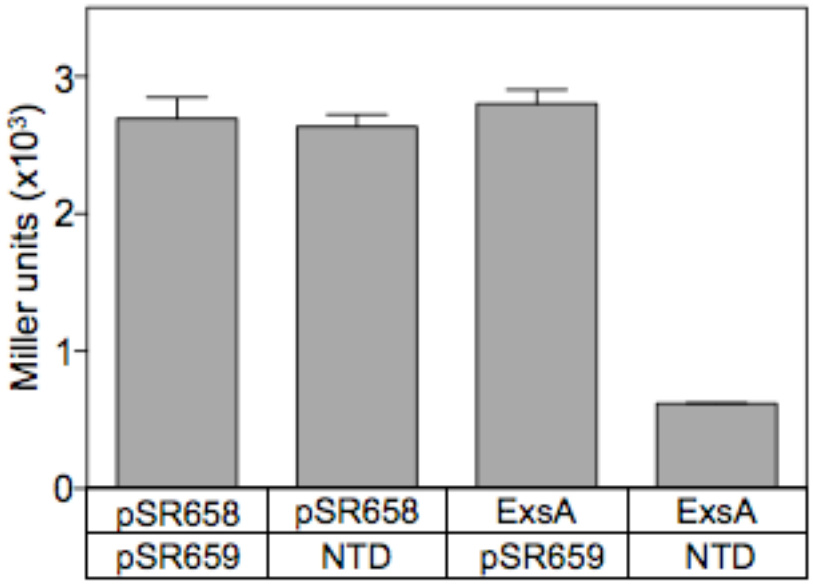

FIG S1. Full-length ExsA interacts with the NTD in the LexA two-hybrid assay. *E. coli* strain SU202 was transformed with vector controls (pSR658 and pSR659), ExsA, and NTD expression vectors. Protein expression was induced by addition of 1mM IPTG. Reporter activity was measured by β-galactosidase activity and reported in Miller units. The reported values are an average of three independent experiments.

Figure S1

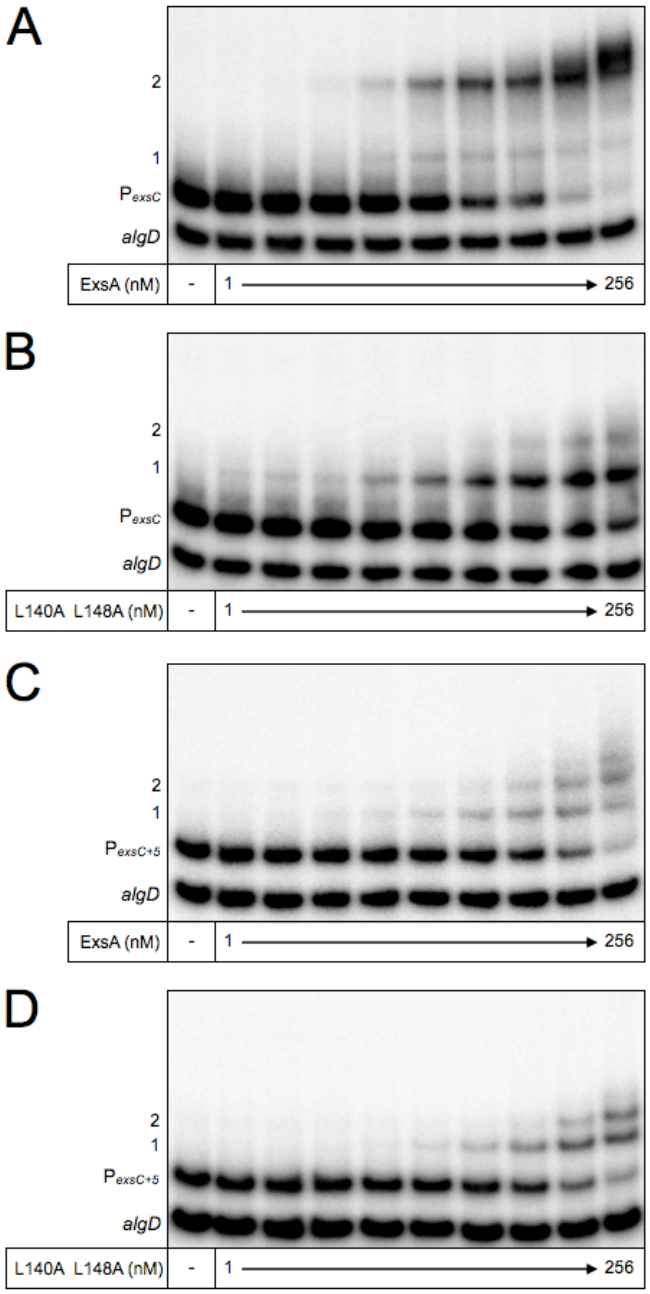

FIG S2. Titration of ExsA and the $ExsA_{L140,L148A}$ double mutant on P_{exsC} and P_{exsC+5}. EMSAs were performed using radiolabeled probes derived from the P_{exsC} (A-B) and P_{exsC+5} (C-D) promoter regions. ExsA and the $ExsA_{L140,L148A}$ (1, 2, 4, 8, 16, 32, 64, 128, 256 nM) were incubated with promoter probes (0.05 nM each) for 15 min at 25°C. Binding reactions were analyzed by native polyacrylamide gel electrophoresis and phosphorimaging.

Figure S2

FIG S3. Native and hybrid promoter sequences used in this study. Binding sites 1 and 2 are underlined and GnC and TGnnA binding determinants that constitute the ExsA consensus binding site are indicated in red. Insertions of 5-10 adenosines between binding sites are shown in bold.

Figure S3

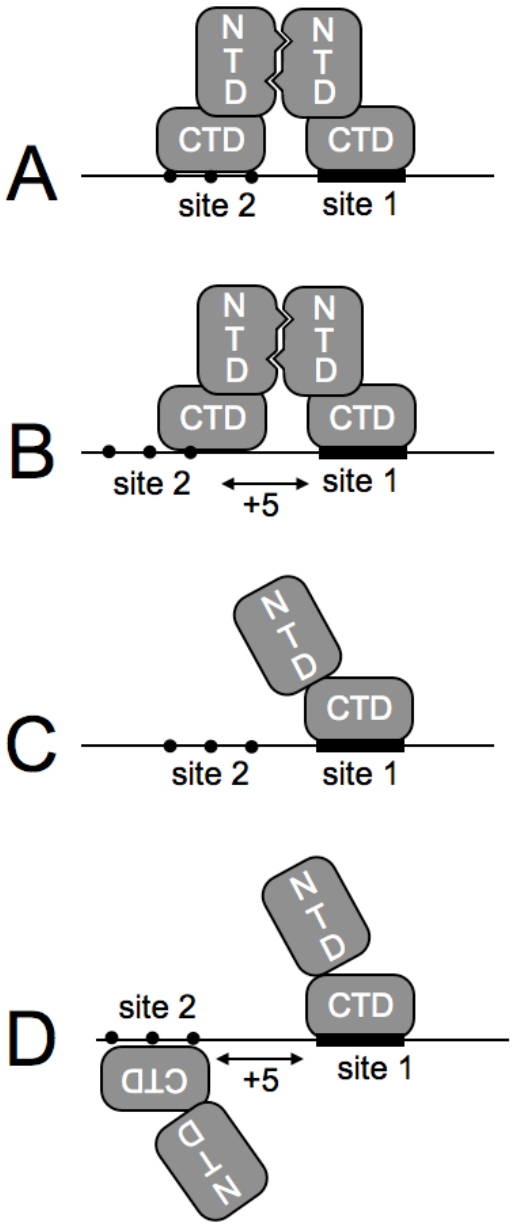

FIG S4. Model for promoters with low affinity site 2 binding sites. (A) ExsA bound to the high affinity site 1 (indicated by a thick solid line) self-associates with and recruits a second monomer to the low affinity site 2 (indicated by the three filled circles) (B) Separation of the high affinity site 1 and low affinity site 2 by 5 bp allows ExsA to occupy site 1. Self-association with the second monomer is stronger than the binding constant of the second monomer for site 2 favoring self-association and non-specific DNA interactions over specific binding of the second monomer to site 2. (C-D) The self-association defect of the L140A-L148A mutants prevents occupation of the second site (C) but that defect is only poorly suppressed by separating sites 1 and 2 by 5 bp owing to the low affinity of ExsA for site 2 (D).

Supplemental information

Self-association is required for occupation of adjacent ExsA-binding sites in *Pseudomonas aeruginosa* T3SS promoters

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Table S1. Bacterial strains and plasmids used in this study.

Bacterial strains	Relevant characteristics	Reference
Pseudomonas aeruginosa		
PA103	Wild-type parental strain	(1)
PA103 *exsA*::Ω	Chromosomal interposon insertion in *exsA*	(2)
PA103 Δ*exsD*	In frame deletion of *exsD*	(3)
Escherichia coli		
DH5α	*recA* cloning strain	(4)
SU101	$P_{sulA\text{-}lacZ}$ reporter strain for monohybrid assay	(5)
SU202	$P_{sulA\text{-}lacZ}$ reporter strain with hybrid operator for two hybrid assay	(5)
BL21 (DE3) Tuner	Protein expression and purification	Novagen

Reporter fusions	Integration plasmid	Experiment	Primer or primer pair	Reference
$P_{exsC\text{-}lacZ}$	mini-CTX-$P_{exsC\text{-}lacZ}$ (-186 to +17)	Fig. 4A, B; 7A; 10	NA	(3)
$P_{exsC+5\text{-}lacZ}$	mini-CTX $P_{exsC+5\text{-}lacZ}$	Fig. 6A, B; 7B; 10	95009717	This study
$P_{exsC+10\text{-}lacZ}$	mini-CTX $P_{exsC+10\text{-}lacZ}$	Fig. 6C, D	116866838	This study
$P_{exoT\text{-}lacZ}$	mini-CTX-$P_{exoT\text{-}lacZ}$ (-179 to +17)	Fig. 2A; 3A; 8A, B; 10	NA	(6)
$P_{exoT+5\text{-}lacZ}$	mini-CTX $P_{exoT+5\text{-}lacZ}$	Fig. 8C, D; 10	115978072	This study
$P_{exsD\text{-}lacZ}$	mini-CTX-$P_{exsD\text{-}lacZ}$ (-152 to +16)	Fig. 2C, 3C	NA	(3)

Expression plasmids	Relevant characteristics	Experiment	Primer or primer pair	Reference
pJN105	Arabinose-inducible expression vector	Fig. 2A, 2C, 3A, 3C, 10	NA	(7)
pSR658	IPTG-inducible expression vector; MCS for in-frame fusions with wild-type LexA DBD	Fig. 2B, 3B, S1	NA	(5)
pSR659	IPTG-inducible expression vector; MCS for in-frame fusions with mutant LexA DBD	Fig. S1	NA	(5)
pAM45	pSR658 derivative with XbaI and SacI in MCS		82218921, 82218920	This study
pET16b	IPTG-inducible protein expression vector that includes and N-terminal His_{10} tag		NA	Novagen
pEB124	Wild-type *exsA* expression vector	Fig. 2A, 3A, 10	NA	(6)

Table S1. (continued) Bacterial strains and plasmids used in this study.

Expression plasmids	Relevant characteristics	Experiment	Primer or primer pair	Reference
pEB109	CTD expression vector		NA	(8)
pAM83	*exsA* M136A	Fig. 2A	83492680	This study
pAM84	*exsA* L137A	Fig. 2A	83492679	This study
pAM85	*exsA* C139A	Fig. 2A	83492678	This study
pAM33	*exsA* L140A	Fig. 2A	82083818	This study
pAM34	*exsA* K141A	Fig. 2A	82083816	This study
pAM35	*exsA* I142A	Fig. 2A	82083815	This study
pAM36	*exsA* E143A	Fig. 2A	82083814	This study
pAM37	*exsA* E144A	Fig. 2A	82083813	This study
pAM38	*exsA* L145A	Fig. 2A	82083812	This study
pAM39	*exsA* L146A	Fig. 2A	82083811	This study
pAM40	*exsA* M147A	Fig. 2A	82083810	This study
pAM41	*exsA* L148A	Fig. 2A	82083809	This study
pAM42	*exsA* F149A	Fig. 2A	82083808	This study
pAM43	*exsA* F151A	Fig. 2A	82083807	This study
pAM44	*exsA* S152A	Fig. 2A	82083806	This study
pAM77	*exsA* L140A, K141A	Fig. 3A	84452013	This study
pAM79	*exsA* L140A, L148A	Fig. 3A, 10	84452022	This study
pAM82	*exsA* K141A, L148A	Fig. 3A	84452014	This study
pSR658 exsA	*lexA*(DBD)-*exsA* fusion	Fig. 2B, 3B, S1	NA	(9)
pAM119	*lexA*(DBD)-*exsA* M136A	Fig. 2B	86360966, 8650714	This study
pAM120	*lexA*(DBD)-*exsA* L137A	Fig. 2B	86360966, 8650714	This study
pAM121	*lexA*(DBD)-*exsA* C139A	Fig. 2B	86360966, 8650714	This study
pAM51	*lexA*(DBD)-*exsA* L140A	Fig. 2B	86360966, 8650714	This study
pAM52	*lexA*(DBD)-*exsA* K141A	Fig. 2B	86360966, 8650714	This study
pAM53	*lexA*(DBD)-*exsA* I142A	Fig. 2B	86360966, 8650714	This study
pAM54	*lexA*(DBD)-*exsA* E143A	Fig. 2B	86360966, 8650714	This study
pAM55	*lexA*(DBD)-*exsA* E144A	Fig. 2B	86360966, 8650714	This study
pAM56	*lexA*(DBD)-*exsA* L145A	Fig. 2B	86360966, 8650714	This study
pAM57	*lexA*(DBD)-*exsA* L146A	Fig. 2B	86360966, 8650714	This study
pAM58	*lexA*(DBD)-*exsA* M147A	Fig. 2B	86360966, 8650714	This study
pAM59	*lexA*(DBD)-*exsA* L148A	Fig. 2B	86360966, 8650714	This study
pAM60	*lexA*(DBD)-*exsA* F149A	Fig. 2B	86360966, 8650714	This study
pAM61	*lexA*(DBD)-*exsA* F151A	Fig. 2B	86360966, 8650714	This study
pAM62	*lexA*(DBD)-*exsA* S152A	Fig. 2B	86360966, 8650714	This study
pAM114	*lexA*(DBD)-*exsA* L140A, K141A	Fig. 3B	86360966, 8650714	This study
pAM116	*lexA*(DBD)-*exsA* L140A, L148A	Fig. 3B	86360966, 8650714	This study
pAM118	*lexA*(DBD)-*exsA* K141A, L148A	Fig. 3B	86360966, 8650714	This study

Table S1. (continued) Bacterial strains and plasmids used in this study.

Expression plasmids	Relevant characteristics	Experiment	Primer or primer pair	Reference
pAM75	*exsA*(NTD) residues 1-180	Fig. 2C, 3C	86360966, 27369454	This study
pAM108	*exsA*(NTD) M136A	Fig. 2C	86360966, 27369454	This study
pAM109	*exsA*(NTD) L137A	Fig. 2C	86360966, 27369454	This study
pAM110	*exsA*(NTD) C139A	Fig. 2C	86360966, 27369454	This study
pAM63	*exsA*(NTD) L140A	Fig. 2C	86360966, 27369454	This study
pAM64	*exsA*(NTD) K141A	Fig. 2C	86360966, 27369454	This study
pAM65	*exsA*(NTD) I142A	Fig. 2C	86360966, 27369454	This study
pAM66	*exsA*(NTD) E143A	Fig. 2C	86360966, 27369454	This study
pAM67	*exsA*(NTD) E144A	Fig. 2C	86360966, 27369454	This study
pAM68	*exsA*(NTD) L145A	Fig. 2C	86360966, 27369454	This study
pAM69	*exsA*(NTD) L146A	Fig. 2C	86360966, 27369454	This study
pAM70	*exsA*(NTD) M147A	Fig. 2C	86360966, 27369454	This study
pAM71	*exsA*(NTD) L148A	Fig. 2C	86360966, 27369454	This study
pAM72	*exsA*(NTD) F149A	Fig. 2C	86360966, 27369454	This study
pAM73	*exsA*(NTD) F151A	Fig. 2C	86360966, 27369454	This study
pAM74	*exsA*(NTD) S152A	Fig. 2C	86360966, 27369454	This study
pAM103	*exsA*(NTD) L140A, K141A	Fig. 3C	86360966, 27369454	This study
pAM105	*exsA*(NTD) L140A, L148A	Fig. 3C	86360966, 27369454	This study
pAM107	*exsA*(NTD) K141A, L148A	Fig. 3C	86360966, 27369454	This study
pET16b *exsA*	source of purified $ExsA_{His}$	Fig. 4, 6, 7, 8, 9	NA	(6)
pET16b *exsA-CTD*	source of purified CTD_{His}	Fig. 4, 6, 8, 9	NA	(8)
pAM145	source of purified $ExsA(L140A,L148A)_{His}$	Fig. 4, 6, 7, 8, 9	42308574, 7736236	This study
pSR659-NTD	l*exA*(DBD)-exsA NTD(1-180)	Fig. S1	5147142, 82234743	This study

Table S2. Primers used in this study.

Primer ID	Name	Primer Sequence
83492680	ExsAM136A	5'-TGCATGAGCATCCGCCGGCCCTCGCCTGCCTGAAG
83492679	ExsAL137A	5'-ATGAGCATCCGCCGATGGCCGCCTGCCTGAAGATC
83492678	ExsAC139A	5'-ATCCGCCGATGCTCGCCGCCCTGAAGATCGAGGAG
82083818	ExsAL140A	5'-CGCCGATGCTCGCCTGCGCCAAGATCGAGGAGTTG
82083816	ExsAK141A	5'-CGATGCTCGCCTGCCTGGCCATCGAGGAGTTGCTG
82083815	ExsAI142A	5'-TGCTCGCCTGCCTGAAGGCCGAGGAGTTGCTGATG
82083814	ExsAE143A	5'-TCGCCTGCCTGAAGATCGCCGAGTTGCTGATGCTC
82083813	ExsAE144A	5'-CCTGCCTGAAGATCGAGGCCTTGCTGATGCTCTTC
82083812	ExsAL145A	5'-GCCTGAAGATCGAGGAGGCCCTGATGCTCTTCGCG
82083811	ExsAL146A	5'-TGAAGATCGAGGAGTTGGCCATGCTCTTCGCGTTC
82083810	ExsAM147A	5'-AGATCGAGGAGTTGCTGGCCCTCTTCGCGTTCAGTC
82083809	ExsAL148A	5'-TCGAGGAGTTGCTGATGGCCTTCGCGTTCAGTCCGC
82083808	ExsAF149A	5'-AGGAGTTGCTGATGCTCGCCGCGTTCAGTCCGCAGG
82083807	ExsAF151A	5'-TGCTGATGCTCTTCGCGGCCAGTCCGCAGGGGC
82083806	ExsAS152A	5'-TGATGCTCTTCGCGTTCGCCCCGCAGGGGCCGC
86360966	Aintstart_Xba5'	5'-CTGCACTCTAGAGCGGGGCATTCAAGGTACGACGGGAAGTGTTGG
83085368	exsA3'Sac	5'-GTGCGAGCTCCTCGTCAGAGCCTGCAATTTGG
27369454	exsA-NTD3'sac	5'-CCACGTGAGCTCTGAGTGCTTCTCCATGAATAGCTGCAGACGCTC
84452013	ExsAL140AK141A	5'-CGCCGATGCTCGCCTGCGCCGCCATCGAGGAGTTG
84452022	ExsAL140AL148A	5'-GCCTGCGCCAAGATCGAGGAGTTGCTGATGGCCTTCGCG
84452014	ExsAK141AL148A	5'-TGCCTGGCCATCGAGGAGTTGCTGATGGCCTTCGCG
82218921	pSR658XbaI5'	5'-GATCGATGGGGATCCTCTAGAGAGATCTGCAGCTGG
82218920	pSR658SacI3'	5'-TACCATATGGGAGCTCGAAGCTTGGCTG
42308574	exsA5'Nde	5'-GTCACATATGCAAGGAGCCAAATCTCTTGG
7736236	exsA3'Bam	5'-GTGAGGATCCGCCGATTCTACTCATGCAGCC
22963127	Pc5'Hind	5'-ACGCAAGCTTATGAAGGACGTCCTGCAGCTCATCC
49188917	Pc3'EcoRI	5'-TGATGAATTCGCCTCCTAAAGCTCAGCGCATGC
31216338	pTforHind	5'-CGAGTAAGCTTGCCAATATCCCATCGGGTTCTCCGCCCCGG
43613960	pT3'EcoRI	5'-CGACGAATTCGACGTCTCCTGATGTTTCCCCGCCAGTCTAGGAACG
95009717	PexsC+5	5'-CAGCGATGTGGCTTTTTTCTTAAAAAAAAAGAAAAGTCTCTCAGTGACAAAAGCG
116866838	PexsC+10	5'-CGATGTGGCTTTTTTCTTAAAAAAAAAAAAAAGAAAAGTCTCTCAGTGACAAAAG
115978072	PexoT+5	5'-CGACGGCCGCCAACAGTAAAAAAAAAAAACCACGGCCAATCCTGATAGGCGGAGG
119836852	EB504+5	5'-CAGCGATGTGGCTTTTTTCTTAAAAAAAAAACCACGGCCAATCCTGATAG
5147142	exsA5'SacI	5'-ATCGGAGCTCATGCAAGGAGCCAAATCTCTTGGC
82234743	exsA-NTD3'Kpn	5'-CCACGGGTACCTCAGTGCTTCTCCATGAATAGCTGCAGACGCTC

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