cAMP and Vfr control Exolysin expression and cytotoxicity of *Pseudomonas aeruginosa* taxonomic outliers

Alice Berry,[a] Kook Han,[b] Julian Trouillon,[a] Mylène Robert-Genthon,[a] Michel Ragno,[a] Stephen Lory,[b] Ina Attrée[a] and Sylvie Elsen[a,#]

Biology of Cancer and Infection, U1036 INSERM, CEA, University of Grenoble Alpes, ERL5261 CNRS, Grenoble, France[a]; Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, USA[b]

[#]Address correspondence to Sylvie Elsen, sylvie.elsen@cea.fr.

Supplemental Material and Methods

Mutagenesis of *exlBA* promoter. Three mutations were introduced into the promoter of *exlBA* directly in the chromosome of the strain IHMA87 *exlA::lacZ*, two abolishing completely the consensus boxes “-10” (“Box -10 mut”) and “-35” (“Box -35 mut”), and the other optimizing the predicted “-35” box to become closer to the consensus one (“Box -35 opt”). To create the mutations, the upstream region and the downstream region flanking the targeted box were amplified using for: 1) “Box -10 mut” : Mut-pexlBA-sF1 / Mut-pexlBA1-new10-sR1 (fragment of 539 pb) and Mut-pexlBA1-new10-sF2 / Mut-pexlBA-sR2 (fragment of 559 pb) ; 2) “Box -35 mut” : Mut-pexlBA-sF1 / Mut-pexlBA1-new35-sR1 (fragment of 515 pb) and Mut-pexlBA1-new35-sF2 / Mut-pexlBA-sR2 (fragment of 583 pb) ; 3) “Box -35 opt” : Mut-pexlBA-sF1 / Mut-pexlBA1-35-sR1 (fragment of 514 pb) and Mut-pexlBA1-35-sF2 / Mut-pexlBA-sR2 (584 pb). The resulting fragments were then cloned into *Sma*I-cut pEXG2 by

Sequence- and Ligation-Independent Cloning (SLIC) (1) and the mutated sequences were introduced into the chromosome by allelic exchange. Each mutation created restriction site (*Sal*I or *Sph*I) allowing selection of mutated genotype. The primers used for PCR are listed in the Table S1.

1. **Li MZ, Elledge SJ.** 2007. Harnessing homologous recombination in vitro to generate recombinant DNA via SLIC. Nat Methods **4:**251-256.

Supplemental Tables

TABLE S1. Primers used in this work

Name	Sequence (5'->3')	Use
Mut-pexlBA-sF1	GGTCGACTCTAGAGGATCCCCTGGTGATGGCGGCTGGCACC	*pexlBA* mutagenesis
Mut-pexlBA-sR2	ACCGAATTCGAGCTCGAGCCCCGACGTAGCCGG CATCGACAT	*pexlBA* mutagenesis
Mut-pexlBA1-new10-sR1	GTCGACGTCGCAGACCTCACCCGTTTG	*pexlBA* mutagenesis
Mut-pexlBA1-new10-sF2	GGTGAGGTCTGCGACGTCGACACGCAAGACACAAAGTTTACATAAC	*pexlBA* mutagenesis
Mut-pexlBA1-new35-sR1	GCATGCCATCCGAGTAAAGAAATTGACCG	*pexlBA* mutagenesis
Mut-pexlBA1-new35-sF2	TTCTTTACTCGGATGGCATGCACGGGTGAGGTCTGCGACTAA	*pexlBA* mutagenesis
Mut-pexlBA1-35-sR1	GTCGACATCCGAGTAAAGAAATTGACCG	*pexlBA* mutagenesis
Mut-pexlBA1-35-sF2	TTTCTTTACTCGGATGTCGACAACGGGTGAGGTCTGCGACTAA	*pexlBA* mutagenesis
Mut-vfr-F1	CTGCAGGCGCCAGCTTAGCACAGGGC	*vfr* deletion
Mut-vfr-R1	GGCGACGCGTCCGGTGACTCAGCGGCCGTCGTCGTCCTCG	*vfr* deletion
Mut-vfr-F2	GTCACCGGACGCGTCGCCC	*vfr* deletion
Mut-vfr-R2	GGATCCTCTCAACCGGGCCGACGTGG	*vfr* deletion
IHMA-Mut-cyaA-F1	CCTCTAGACCGACGCTGTTCCCTAGTCC	*cyaA* deletion
IHMA-Mut-cyaA-R1	CTGTCGGTTCATGGGCGTCC	*cyaA* deletion

IHMA-Mut-cyaA-F2	GGACGCCCATGAACCGACAGCAGGCGCTGCTGGAGCAATGA	*cyaA* deletion
IHMA-Mut-cyaA-R2	GGCTCGAGATGCCTTCCTGTGCCTGCTG	*cyaA* deletion
IHMA-Mut-cyaB-F1	CCTCTAGAGTGCTCTTCCACGCGCTGGC	*cyaB* deletion
IHMA-Mut-cyaB-R1	GAGGGTGGGCTTCATGCGCT	*cyaB* deletion
IHMA-Mut-cyaB-F2	AGCGCATGAAGCCCACCCTCTACGTCGAGCACGAACTGCCC	*cyaB* deletion
IHMA-Mut-cyaB-R2	GGCTCGAGCCTGGTGATGCTCGAAGCC	*cyaB* deletion
Vfr-binding-F1	GGATCCTACGGCATGCATCTCGATGTC	p*exlBA* Vfr binding site mutation
Vfr-binding-R1	GCCTCTAGACTGATTCACGAAAGTTTGGCG	p*exlBA* Vfr binding site mutation
Vfr-binding-F2	CTTTCGTGAATCAGTCTAGAGGCGTTTCTTCGTCCAGTCAGCAAC	p*exlBA* Vfr binding site mutation
Vfr-binding-R2	CTCGAGGCAATTGGCGTTGGCGTTCCT	p*exlBA* Vfr binding site mutation
IHMA-Comp-vfr-F	CCGGATCCCGGCCTCGAGGAAGGCCTCGCAGC	*vfr* complementation
IHMA-Comp-vfr-R	CCAAGCTTCTGACTGATCCGCGCTGTCGA	*vfr* complementation
Comp-cyaA-sF	GATATCGAATTCCTGCAGCCCGGCCGAACACCTGCTCGAGC	*cyaA* complementation
Comp-cyaA-sR	CTCTAGAACTAGTGGATCCCCCGATAGCCATGGATTACGTCCCT	*cyaA* complementation
IHMA-Comp-cyaB-sF	GATATCGAATTCCTGCAGCCCTTTCGCCGAGTTCTACCCCTAT	*cyaB* complementation
IHMA-Comp-cyaB-sR	CTCTAGAACTAGTGGATCCCCCGAGCAATCCTGGCGGGCCTC	*cyaB* complementation
SLIC_pEXG2_exlBA'_F	TGCAGGTCGACTCTAGAGGATCCCCAGCGCGGTCTGGAGTCTCG	*exlA-lacZ* fusion
SLIC_exlBA'_lacZ_R	AAGTTAAAATGCCGCGCCCCTACCTTCTATGCATGAGAACCTCTTCG	*exlA-lacZ* fusion
SLIC_lacZ_F	AGGTAGGGGCGCGGCATTTT	*exlA-lacZ* fusion
SLIC_lacZ_R	TTATTTTTGACACCAGACCAACTG	*exlA-lacZ* fusion
SLIC_lacZ_exlA'_F	CCAGTTGGTCTGGTGTCAAAAATAAGACAATCCTGTCTTCCACCTC	*exlA-lacZ* fusion
SLIC_exlA'_pEXG2_R	AGGTACCGAATTCGAGCTCGAGCCCTCGAGCTCGGTCGACGCCT	*exlA-lacZ* fusion
Purif-vfr-NdeI-F	GGCATATGGTAGCTATTACCCACACACCCA	Vfr over-production
Purif-vfr-BamHI-R	GGGGATCCTTCAGCGGGTGCCGAAGACCA	Vfr over-production
R_exlB+60	CAGCAGCAGGGCTCGGCAG	circularization

F_exlB+4	CGTACCGCTCTACCGAATCATC	circularization
R_exlB+3	CACGCGGCATCCTTCATGTATC	circularization
rpoD-F3	CTGTTCATGCCGATCAAGCTG	RTqPCR Reference gene
rpoD-R3	AACGCTGTCGACCCACTTCTC	RTqPCR Reference gene
exlB-F	CCTATGGCTACTGGACCTACA	RTqPCR
exlB-R	AGGTAGCTGTCGACATCCTTG	RTqPCR
exlA-F	CGCTGAAGGACAAGCTGGAA	RTqPCR
exlA –R	CATTACGGTCGATGCCGTTC	RTqPCR
5'Cy5-p*exlBA*_EMSA_F	CCAGTCGCGACACGCCAAACTTTCGTGAATC AGTTCACAGGCGTTTCTTCGTCCAGTAG	EMSA
p*exlBA*_EMSA_F	CCAGTCGCGACACGCCAAACTTTCGTGAATC AGTTCACAGGCGTTTCTTCGTCCAGTCAG	EMSA
p*exlBA*_EMSA-R	CTGACTGGACGAAGAAACGCCTGTGAACTG ATTCACGAAAGTTTGGCGTGTCGCGACTGG	EMSA
5'Cy5-p*exlBA*_mut_EMSA_F	CTGACTGGACGAAGAAACGCCTGTGAACTG ATTCACGAAAGTTTGGCGTGTCGCGACTGG	EMSA
p*exlBA*_mut_EMSA_F	CCAGTCGCGACACGCCAAACTTTCGTGAATC AGTCTAGAGGCGTTTCTTCGTCCAGTCAG	EMSA
p*exlBA*_mut_EMSA-R	CTGACTGGACGAAGAAACGCCTCTAGACTG ATTCACGAAAGTTTGGCGTGTCGCGACTGG	EMSA

A

GTCAATTTCTTTACTCGGATGTAACAAACGGGTGAGGTCTGCGACTAATCTACGCAAG**A**CACAAAGTT

-35 -10

consensus :	TTGACA	$N_{16/18}$	TATAAT	$N_{5/8}$	+1 (A/G)
exlBAp :	TAACAA	N_{18}	TAATCT	N_7	+1 (A)
Box -10 mut :	TAACAA	N_{18}	**gtcgac**		
Box -35 mut :	**gc**A**tgc**	N_{18}	TAATCT		
Box -35 opt :	T**cgac**A	N_{18}	TAATCT		

B

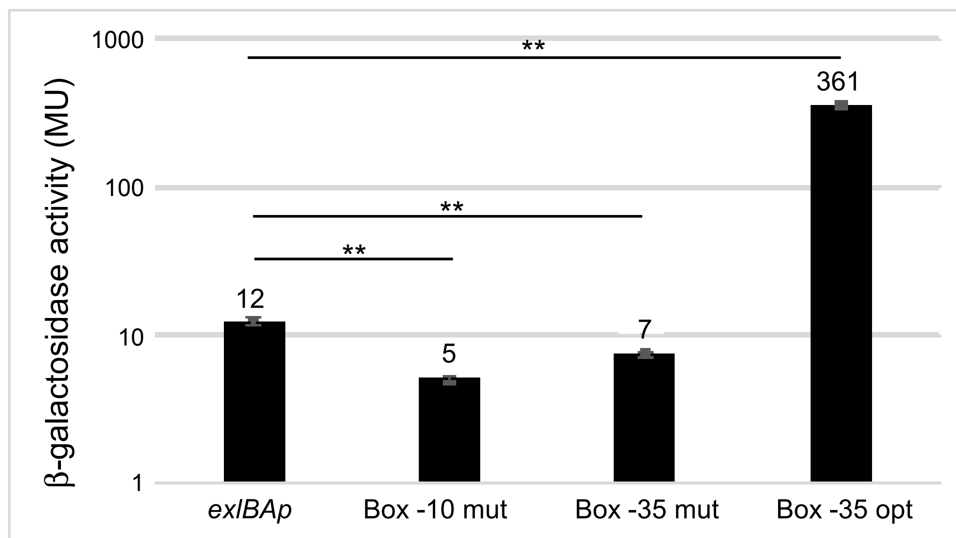

FIG S1 Definition of the *exlBA* promoter. (A) DNA sequence of the 5' region of exlB, with the nucleotide "A" identified as the TSS in bold and pinpointed by an arrow. The putative "-10" and "-35" boxes are underlined and compared to the consensus "-10" and "-35" sequences, with the conserved nucleotides underlined. The mutations introduced in the chromosome of the IHMA87 *exlA::lacZ* strain are indicated with the mutated bases in bold lower cases. The selected changes either reduce (Box -10 mut and Box -35 mut) or increase (Box -35 opt, "opt" for "optimal") the homology to the consensus. (B) β-galactosidase activities of IHMA87 *exlA::lacZ* strain with the indicated *exlBA* sequences. Strains were grown in LB medium at 37°C to an OD_{600} of 1.5 then β-galactosidase activity was measured. The experiments were performed in triplicate and the mean values of the β-galactosidase activities are indicated on the top of the histograms (in Miller Units) while the error bars indicate the standard deviations. The p-value ($p<0.01$) is determined using Mann-Whitney U test and indicated by two stars when the difference with the wild-type *exlBA-lacZ* fusion is statistically supported.

Note that the implication of another putative -35 box (underlined with dashed line, panel (A)) closer to the consensus was ruled out as its modification from GTAACA to GTCGAC ("box -35 opt") led to a higher activity of *exlBAp*. The poor matches of the identified "-10" and "-35" boxes to consensus might explain the low *exlBA* transcriptional activity measured in the IHMA87 strain harboring the native promoter fusion and the mild impact of introduced mutations. On the other hand, the change of the putative "-35" box to fit better the consensus led to strong increase (30 fold) of *exlBAp* activity.

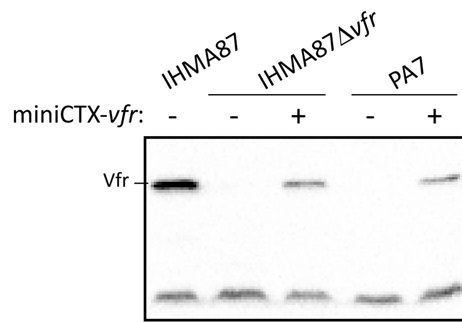

FIG S2 Vfr synthesis in wild-type and complemented strains. Western blot of the cytosolic fractions of the indicated strains grown in low calcium condition (EGTA/$MgCl_2$). The upper band corresponds to Vfr, while the lower band is a non-specific cross-reacting protein used as a loading control. The amount of Vfr observed in the complemented strains is lower compared to the parental strain IHMA87, probably due to the ectopic location (*att* site) of the own-promoter driven *vfr* gene.