

SUPPLEMENTAL MATERIAL

**Transcriptional regulation of the *ecp* operon by EcpR, IHF and H-NS in
attaching and effacing *Escherichia coli***

**Verónica I. Martínez-Santos¹, Abraham Medrano-López¹, Zeus Saldaña²,
Jorge A. Girón² and José L. Puente^{1*}.**

Table S1. Oligonucleotide primers used in this study.

Primer	Sequence 5'-3'	Use
hns-H1P1	CACCCCAATATAAGTTTGGAGTACTACAATG AGCGAAGCTGTAGGCTGGAGCTGCTTCG	Mutagenesis of <i>hns</i>
hns-H2P2	GATTTTAAGCAAGTGCAATCTACAAAAGATTA TTGCTTCATATGAATATCCTCCTTAG	Mutagenesis of <i>hns</i>
hns-M	TGCTGCGAGCTCATCGGTGTAA	Screening of mutants
hns-O	GCCTATCATATGAAAGGGAAG	Screening of mutants
himA-H1P1	ATGGCGCTTACAAAAGCTGAAATGTCAGAATA TCTGTTTGTATTGTAGGCTGGAGCTGCTTCG	Mutagenesis of <i>himA</i>
himA-H2P2	CTTTTTAGTTAGATCAGTACTCCGTTTTGG GCGA AGCGTTCATATGAATATCCTCCTTAG	Mutagenesis of <i>himA</i>
himA-F	AAGGGTGTTGCGGAGGGGTAT	Screening of mutants
himA-R	CAAACTGACAGGCATAATAA	Screening of mutants
G64	GCCTGGAGTTTACTGAACCAACTTATATAATT TTGAGTACAGCATATGAATATCCTCCTTAG	Mutagenesis of <i>ecpR</i>
G65	AAAGTAGTGACATGGCAAATGATTACAGCAG GGACTATGAGTGTAGGCTGGAGCTGCTTCG	Mutagenesis of <i>ecpR</i>
G92	ACCTATATTGATATGTGCTACG	Screening of mutants
G93	CGTTACCAGAGCTATTGCCAG	Screening of mutants
ecpR-600F	CAAAGAGGGGATCCTTCCTGTGAC	pecpR-2
ecpR-500F	GTTTAATTTTGGATCCCTTATTGG	pecpR-3
ecpR-400F	CTTTAATGAGGATCCATGATAGTT	pecpR-4
ecpR-350F	GTTTTTTTACGGATCCATTACACA	pecpR-5
ecpR-300F	TCAGGGCAAGGATCCTGGCTAATA	pecpR-8
ecpR-200F	CTTTTTCTTCGGATCCAAAAGCA	pecpR-9
ecpR-190F	ATATGGATCCAACCATGGAATTCATTTTC	pecpR-10
ecpR-160F	CTGTGGATCCATTGACACTCATCAGGAA	pecpR-11
ecpR-130F	ACATGGATCCAAACCTAATCGAAATAAT	pecpR12
ecpR-100F	TAAACTGGATCCCGTTTAAACCTA	pecpR-13
ecpR-50F	ATTTACTTCGGATCCACTAAAGAA	pecpR-14
ecpR-1R	TGGGGAGACAAGCTTTATTTATCA	<i>ecpR</i> fusions
ecpA-270F	TGGCCTATGGGATCCATGGCAGGT	ecpA-270

ecpA-180F	CTCAGGGAAGGATCCCTAAATCGA	ecpA-180
ecpA-80F	ATATAAGTTGGATCCGTAAACTCC	ecpA-80
ecpA-Rev	CCGTTACCAAAGCTTTTGCCAGAA	<i>ecpA</i> fusions
ecpR-Rev	TCATAGTCCCTGCTGTAATCA	Primer extension
D60A-F	AAATCAGAAAAGCTTTCGTGTTTAT	pT3-D60A
D60A-R	ATAAACACGAAAGCTTTTCTGATTT	pT3-D60A
G159A-F	TGACGGCTCAGGCAATGCTGCCTAA	pT3-G159A
G159A-R	TTAGGCAGCATTGCCTGAGCCGTC	pT3-G159A
T175A-F	TGTAGTGTGAAGGCAGTGTATACCC	pT3-T175A
T175A-R	GGGTATACTGCCTTCACACTACA	pT3-T175A
V176A-F	GTGTGAAGACAGCGTATACCCATCG	pT3-V176A
V176A-R	CGATGGGTATACGCTGTCTTCACAC	pT3-V176A
K186A-F	AATGCAGAGGCCGCGCTGTACTION	pT3-K186A
K186A-R	TTTGAGTACAGCGCGGCCTCTGCATT	pT3-K186A
G10	GAAGATCTATGGAATGTCAAACCGTTCT	RT-PCR
G85	CGCGAATTCTAACTGGTCCAGGTCGCGTCG	RT-PCR
G84	CGCGGATCCATGAAAAAAGGTTCTGGC	RT-PCR
G12	CGAAGCTTCTATTTACGGGAATGAACTT	RT-PCR
EcpR-EcoR1F	CAGGTTTGGGAATTCGTGACATGG	Cloning of MBP-EcpR
6HEcpR-R	AACATAACAAGCTTGGAGTTTACTG	Cloning of MBP-EcpR
ecpR-Nco-1F	GGTTTGGACCATGGTGACATGGCAAATG	Cloning of EcpR-MycHis
ecpR-H3-R	CTTGCCTGGAAGCTTCTGAACCAAC	Cloning of EcpR-MycHis
pKK-8-BHI-F	GGAATTCTCGGGGAT	<i>In vivo</i> footprinting
ecpR-3R	AATGAATTCCATGGTTAAGTC	<i>In vivo</i> footprinting
EBS-F	ACTATTCCTAACACCTCCTTTACCCTGGACTG GCTAATATAAAATG	pecpR-4m3
EBS-R	CATTTTATATATGCCAGTCCAGGGTAAAGGAG GTGTTAGGAATAGT	pecpR-4m3
upEBS-F	ACACATTAAGACTCGGAATAACACCTCAGGG	pecpR-4m1
upEBS-R	CCCTGAGGTGTTATTCCGAGTCTTAATGTGT	pecpR-4m1
ecpRm4-F	CACCTCAGGGCAAAGGGAATGGCTAATATAA AATGC	pecpR-4m4
ecpR-m4R	GCATTTTATATTAGCCATTCCCTTTGCCCTGA GGTG	pecpR-4m4
EBSm2-R	TTTATATTAGCCAGGAACGGGTCCCTGAGGT GTTAGGAAT	pecpR-4m2
pKKAp59F	CTGTTGTTTGTCCGGTGAACG	pecpR-4m2
EBSm2-F	ATTCCTAACACCTCAGGGACCCGTTCCCTGGC TAATATAAA	pecpR-4m2
pKK-8-H3R	GTTTTGTCCTACTCAAGC	pecpR-4m2
IHFBS-F	TCTTCTTATCAAAAAAGGGATCCTTTCATT TTTTGTAAAT	pecpR-4IRm1
IHFBS-R	ATTTACAAAAAATGAAAGGATCCCTTTTTTGT AAGAAGA	pecpR-4IRm1

IHFm-F	CTTTTTCTTCTTATCAAAGGGGCGCACCGGC ATTTTTGTAAATATTG	pecpR-4IRm2
IHFm-R	CAATATTTACAAAAAATGGTGCGCCCTTTTG ATAAGAAGAAAAAG	pecpR-4IRm2
ecpR- 350AC	TTTTACGGATCCATTACACAACACAGGCCTCA GATTCCTAACACC	pecpR-5m

Fig. S1. EcpR (also called MatA) belongs to the family of proteins containing a LuxR_C-like DNA-binding HTH domain. **A)** The amino acid sequence multialignment of EcpR from EHEC EDL933 (AAG 54619.1), NarL from *Pseudomonas aeruginosa* PAO1 (NP_252568.1), FixJ from *Sinorhizobium meliloti* (CAA79898.1), UhpA from EHEC EDL933 (NP_290304.1), GerE from *Bacillus subtilis* (CAA11701.1), MalT from *E. coli* K-12 (AAA83888.1), LuxR from *Vibrio fischeri* ES114 (YP_206883.1), and RmbA from *S. enterica* subsp. *enterica* serovar Typhimurium (AAD16953.1), was done using the ClustalW sequence alignment program from the European Bioinformatics Institute (EMBL-EBI). Identical amino acids are boxed, and similar amino acids are shaded in gray.

Fig. S2. Global regulators involved in *ecp* regulation. **A)** Expression of the *ecpR-1 cat* transcriptional fusion in wild type EPEC E2348/69 and its Δhns , Δfis , Δhha , $\Delta himA$ and $\Delta stpA$ isogenic mutants. CAT specific activity was determined from samples obtained from static DMEM cultures grown at 30°C. The bars represent the activity of three independent assays with duplicates. Asterisks (*) correspond to *P* values < 0.0001 between the wild type strain and the mutants. **B)** Western blot with the anti-ECP antibody of whole cell extracts of EPEC E2348/69 and its isogenic Δhns and $\Delta himA$ mutants transformed with vector pBAD/MycHis A or plasmid pGTG expressing EcpR (Table 1). DnaK was detected as a loading control using a monoclonal anti-DnaK antibody.

Fig. S1

```

NarL  LRLAAELDPDMILLDLAMKGMNG-----LDTLRALREAGVDARIVFTSDDKGDVFN 102
FlxJ  LAFAPDVRNGVLVTLRNPDMSG-----VELLRNLGLKINIPSIITGHGDVPMAVE 92
UtpA  LAGLPGRGVQVCKDIIMPDISG-----LILLSQLPKG---MATIILSHDSPAIVEQ 89
GexE  ----- 1
MalT  LRLMSDLNRNLLLNLQLYWQAGRKSDAQRVLDALNLAARTGFISHPMIEGEAMAQQIRQ 780
LusR  YYDDAGLLEYDFWVDYKSHHS PINWVFEKTKKESRNVIKEAQESGLITGFSFPIHT 128
EcpR  IDRLIYLSLEKIRKDFVINLNT-----SLTEFIRDNENLSAMKGGQVVLIAARK 97
RsbA  LDRHNHLSGFIYVDFSPNLR-----FTLQMADSLADSGSHIVLISDRS 102

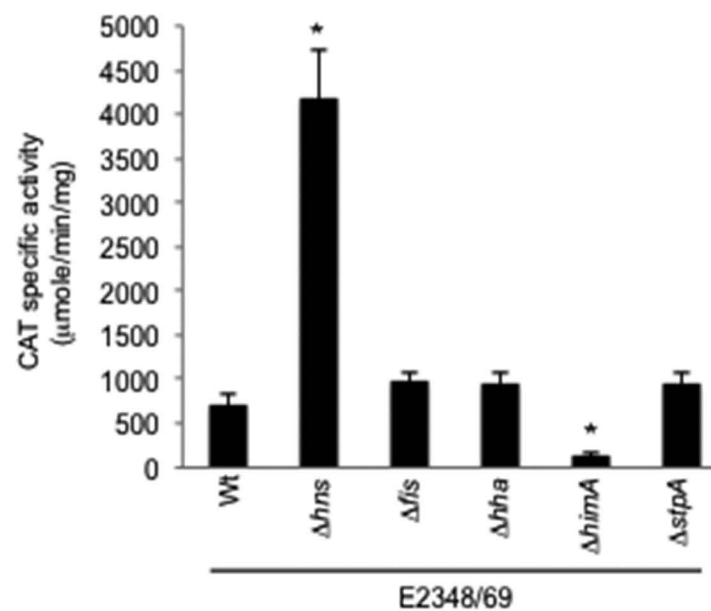
NarL  VLRAGADGVLLKDMEPERLEHIRQAATGQMTSPQLTQILAQALRGDDRSKSLDELTER 162
FlxJ  AMKAGAVDFIEKPFEDTVII EAIERASEHLVAAEADVDANDIRAR-----LQTLSEK 145
UtpA  ALNAGARGFLSKRCSFDELIAAVHTVATGCCYITPDIAIKLASGRQ-----DPLTER 141
GexE  -NKE--KEFSK--P-----LTER 15
MalT  LIQLNTLPLELQHRQRLEINQHRRHKFAHF DENFVERLLNHPEVPELIR-TSPLEK 839
LusR  ASNGFGMLSFAHSDKDIYTDSLFLHASTNVPLLPSLVINYQKINTTRKSD--SILTER 186
EcpR  SEALANYWYNSNIRGVVYAGLSRDIRKELAYINGRFLRKDIKKD-----KITDR 148
RsbA  LTPLANYWIKSNKIQGIYSDDDDIVQQQKMHRLFTGRLANSKRG-----RTLNVT 154

NarL  ERQILRQIANGYSKMIARKLITTEGTVKVVHKKRVLHKLGRSRVEAVMAVENDLV--- 219
FlxJ  ERQVLSAVVAGLEPKSIAYDLIISPTVEVHBRANVMAKAKSLPHLVRMAGGFGPS- 204
UtpA  ERQVREKLAQCNAKELAAELGISPKTVHVBANLMEKLGYSNDVELARRMFCM----- 196
GexE  ERVVFELIVQDKTKELASELHISEKTVRNHISNAOKLGVKCRSQAVVELRMGEL-EL 74
MalT  EKQVGLLYSGYSNECIAGELEVAATIKTHIRNYQKLGFAHRQDAVQHAQQLLKMGY 899
LusR  EKECLAWASEGKSTMDISKILGCEHTVTFHLENTQMKINTTRCQSSKALTGAINCP 246
EcpR  EMEIIRMTAQQMLPKSLARIENGCVKIVYTHARNAEAKLYSKLYKLVQ----- 196
RsbA  EFILKRFVSGIETQIVNIDIDIKLYVHLLRENKLGHSIHKIISNIL----- 205

```

Fig. S2

A



B

