Cell Host & Microbe, Volume *22*

Supplemental Information

A Two-Component System Regulates *Bacteroides fragilis* Toxin to Maintain Intestinal Homeostasis and Prevent Lethal Disease

Aaron L. Hecht, Benjamin W. Casterline, Vivian M. Choi, and Juliane Bubeck Wardenburg

Supplemental figures and tables

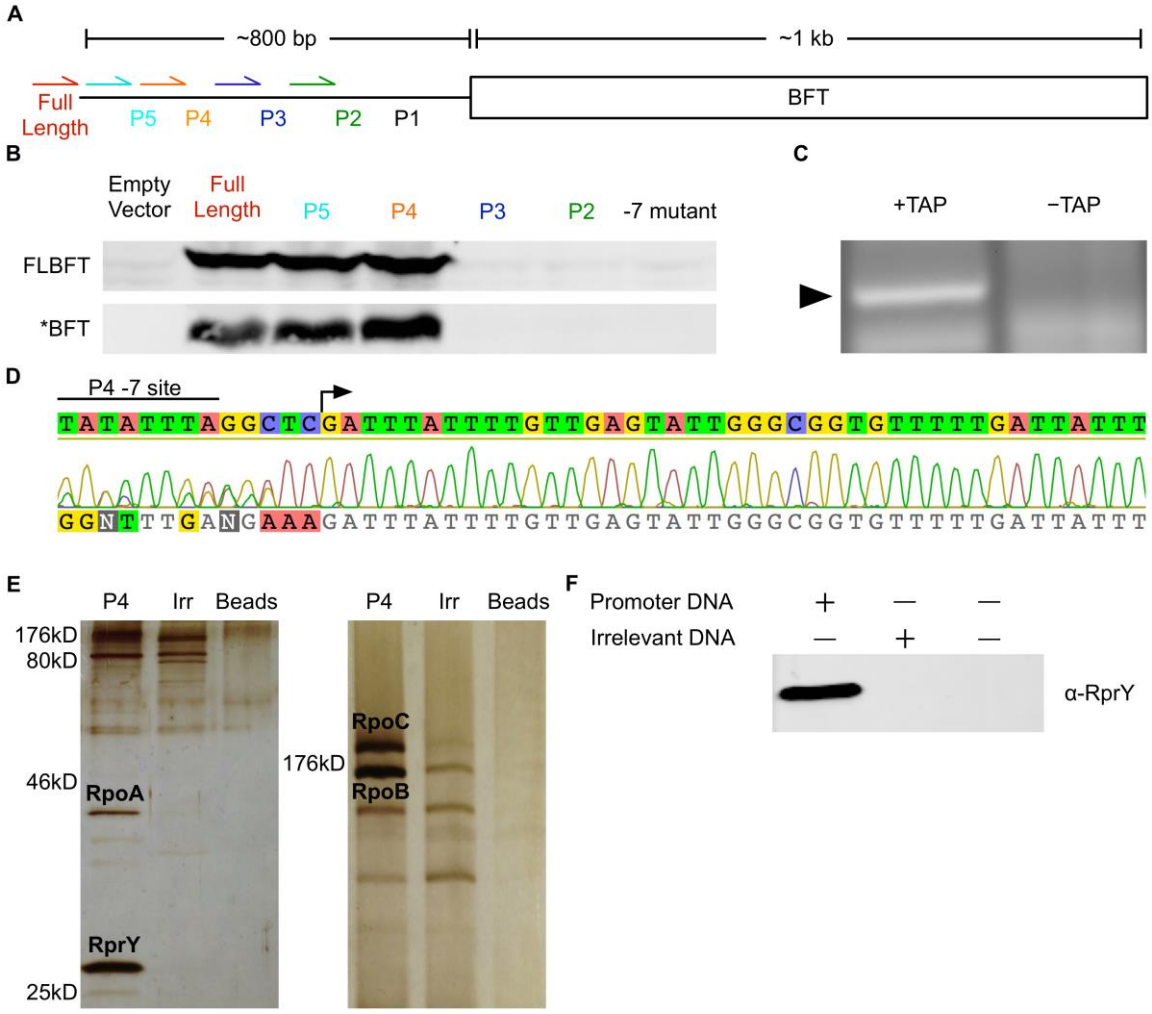

Figure S1. The putative promoter P4 is the RNA polymerase binding site for toxin transcription.

(A) Schematic of the *bft* gene with five putative promoters (P1-5) identified from homology to approximate sigma factor binding site consensus sequence (Franco et al., 2002). Upstream primers used for cloning region are shown at approximate scale.

(B) Immunoblot against anti-6xHis tag of BFT from cell pellet (FLBFT) and supernatant (BFT*) from overnight culture of ETBF encoding plasmid-borne BFT with truncations of upstream region, matching the color scheme of A. Mutation of the P4 site in the -7 location was tested in the context of the full ~800bp upstream region. Results are representative of three independent experiments.

(C) Agarose gel electrophoresis of 5'RACE reaction with and without Tobacco Acid Pyrophosphatase (TAP). The band identified as specific to the +TAP lane is indicated (arrowhead). Results are representative of two independent experiments.

(D) DNA sequence of the region upstream of the *bft* gene, including the and P4 -7 site (upper) and Sanger sequencing results from the band indicated in part C (lower). Mismatch is denoted by color in the lower sequence and indicates the presence of the adapter sequence. The arrow demonstrates the transcriptional initiation site.

(E) DNA pulldown was performed with *bft*-promoter DNA (P4) or irrelevant control DNA (Irr) as bait from ETBF ATCC 43858 lystate. The elutions were run on 15% (left) or 5% (right) polyacrylamide gels and silver stained. Multiple specific bands appeared in the promoter lane and not the irrelevant DNA or no DNA (Beads). Bands were excised and sent for mass spectrometry peptide analysis. The identified protein is labeled above (RpoA, RprY, RpoC) or below (RpoB) each band. Gels are representative of three independent experiments, with identified bands appearing in each repeat. Mass spectrometry was performed once for each identified band, with the results displayed in Table S1.

(F) Immunoblot performed with anti-RprY serum after DNA-pulldown of ETBF lysate with P4, Irr or no DNA. Results are representative of three independent experiments

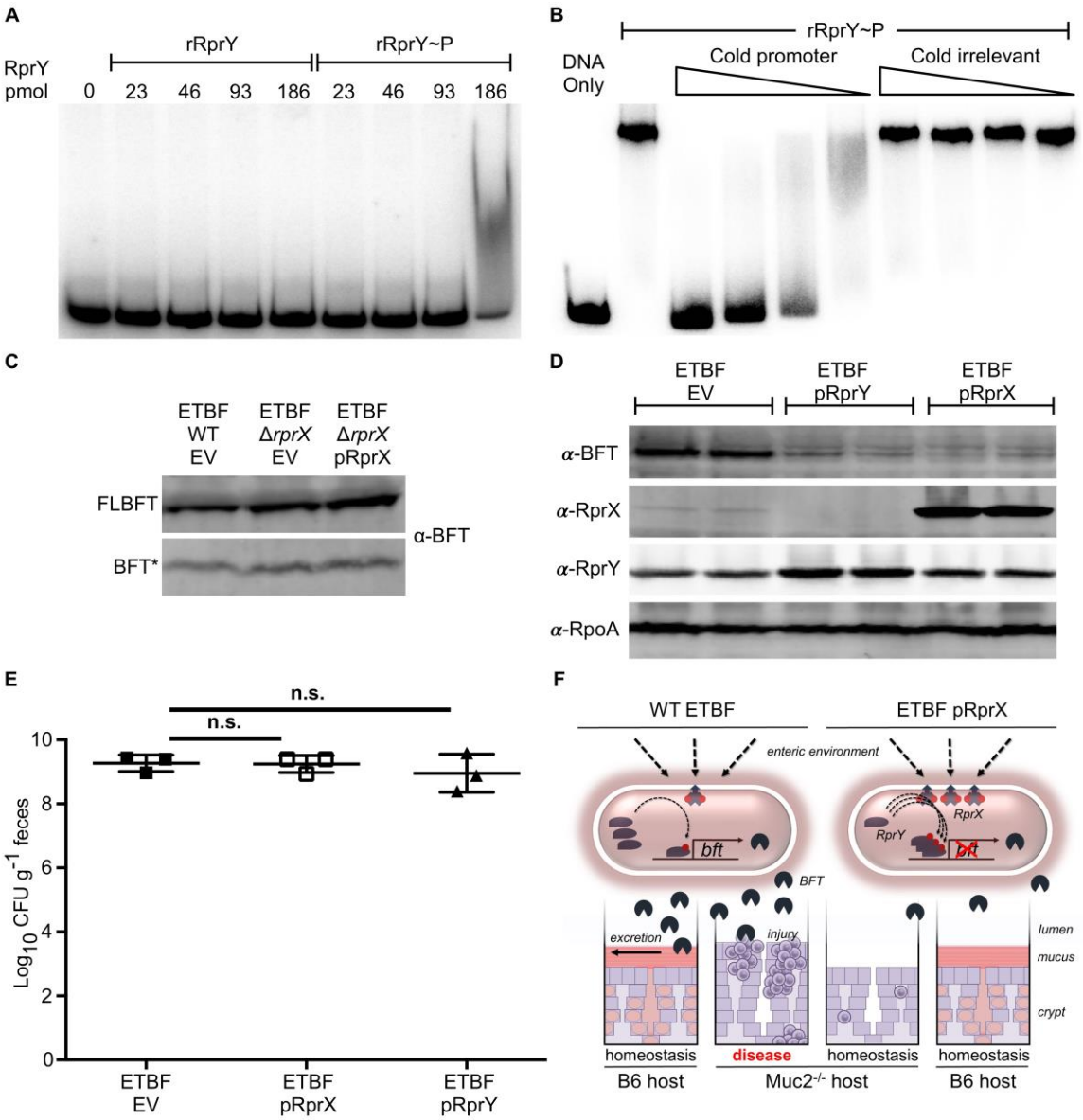

Figure S2. RprY binds specifically to the *bft* promoter sequence, but deletion of RprX in ETBF has no effect on BFT expression *in vitro*.

(A) EMSA was performed with labeled irrelevant DNA and increasing concentrations of rRprY with (rRprY~P) or without acetyl-phosphate treatment. Results are representative of three independent experiments.

(B) EMSA of ^{32}P labeled *bft* promoter with rRprY~P followed by competition with cold promoter DNA or irrelevant DNA. The high concentration of cold competitor is a 1000x, tested to a low of 100x. Results are representative of three independent experiments.

(C) *RprX* was deleted via allelic exchange in ETBF (ETBF Δ*rprX*). Empty vector or *rprX* complement vector was transformed into the mutant strain to generate ETBF Δ*rprX* EV and ETBF Δ*rprX* pRprX, respectively. Immunoblot with anti-BFT serum was performed on overnight cultures to test for altered toxin production. Results are representative of three independent experiments.

(D) RprY or RprX were overexpressed downstream of the GAPDH promoter on a plasmid in ETBF ATCC 43858. Detection of various proteins was performed via immunoblot, utilizing anti-BFT, RprX, RprY and RpoA (loading control) antibodies, from the cell pellet of ETBF encoding the overexpression constructs. Results are representative of three separate experiments.

(E) WT mice were orally gavaged with ETBF ATCC 43858 encoding empty vector (ETBF EV) or vectors overexpressing RprX (ETBF pRprX) or RprY (ETBF pRprY). Two days after colonization, fecal CFU was quantified. Results are representative of three independent experiments; data are represented as mean ± SD; p-value was calculated using one-way ANOVA with Dunnett's multiple comparisons test comparing denoted groups to ETBF EV; n.s., not significant.

(F) Homeostasis requires an interaction between host and ETBF factors. Signals from the intestinal environment are transduced through RprX via autophosphorylation (red), and subsequent phosphotransfer to RprY, suppressing *bft* expression. ETBF pRprX produces decreased BFT relative to WT ETBF, protecting Muc2-deficient hosts from lethal disease and restoring homeostasis.

Table S1. Mass spectrometry peptide analysis of extracted polyacrylamide gel bands

Band	**Protein Identity**	**Peptide Fraction**	**Coverage**
1	Transcriptional Regulatory Protein (RprY)	33/39	52.54%
2	RNA polymerase Alpha (RpoA)	4/4	7.58%
3	RNA polymerase Beta (RpoB)	21/21	11.02%
4	RNA polymerase Beta' (RpoC)	27/28	15.70%

Table S2. Primers

Name	Function	Sequence
Promoter full length	Promoter truncation	GGATCCAGTGGAAATGGGATGAGT
Promoter P5	Promoter truncation	GGTACCATGCCAAGAAAATATTGTTTTAATTA
Promoter P4	Promoter truncation	GGTACCGGCTCGATTTATTTTGTTGAGTA

Promoter P3	Promoter truncation	GGT ACC ATT ATT TAT TTT AAT CGT TTT TAA TAA A
Promoter P2	Promoter truncation	GGTACCTTACTGTCCATAACTGTATTCC
Promoter P4 -7 For	-7 site mutation	CACCTATA**AAAT**GGCTCGATTT
Promoter P4 -7 Rev	-7 site mutation	AAATCGAGCC**ATTT**TATAGGTG
Promoter Rev 6xHis	Promoter truncation	GGTACCCTAGTGATGATGATGATGATGATCGCCATCTG CTATTTC
GAPDH For	Const. promoter	ATACGCGGTACCCTTTTTTTATATTTAATATGAATTTAAT
GAPDH RprY fusion Rev	pRprY	GTTTCTCGTCCATATCAATCATTTTGTTTAAACTTTTAAG GGTTTTA
GAPDH RprY fusion For	pRprY	TAAAACCCTTAAAAGTTTAAACAAAATGATTGATATGGA CGAGAAAC
RprY Rev	pRprY	GGATCCTTATGATTCGGGTTCGGGGG
GAPDH RprX fusion Rev	pRprX	ATCCAGATTGTTGACTTTTTCATTTTGTTTAAACTTTTAA GGGTTTTA
GAPDH RprX fusion For	pRprX	TAAAACCCTTAAAAGTTTAAACAAAATGAAAAAGTCAAC AATCTGGAT
RprX Rev	pRprX	GGATCCTCAATCATTTTTTAATAAAGGTAATG
RprX KO US For	Δ*rprX*	GGATCCAATTTAAGATTAACGTGAAGGGT
RprX KO US Rev	Δ*rprX*	TTTTAATAAAGGTAATGCAATAATAAATATCCAGATTGTT GACTTTTTCAT

RprX KO DS For	Δ*rprX*	ATGAAAAAGTCAACAATCTGGATATTTATTATTGCATTACCTTTATTAAAA
RprX KO DS Rev	Δ*rprX*	GGATCCACACACACATGAAAGGCTTATT
RprY For	RprY-pET28b	CCATGGATGATTGATATGGACGAGAAAC
RprY 6xHis Rev	RprY-pET28b	GCGGCCGCTTAGTGGTGGTGGTGGTGGTGTGATTCGGGTTCGGGGGTG
RprX For	RprX-pET28b	CCATGGATGGTCTTCCGTCAGAAGAAGTTG
RprX 6xHis Rev	RprX-pET28b	GCGGCCGCTCAGTGGTGGTGGTGGTGGTGATCATTTTTTAATAAAGGTAATGC
5’RACE *bft* sequencing	5’RACE	GATGCACGATGTGATTCCACA
5’RACE *bft* amplification	5’RACE	GATACTCATCCCATTTCCACT
P4 Biotin For	DNA pulldown/ EMSA	5’Biotin-ATGCCAAGAAAATATTGTTTTAATTA
P4 Rev	DNA pulldown/ EMSA	CAACCGATGCCTGTCTGCT
Irr Biotin For	DNA pulldown/ EMSA	5’Biotin-ATGAGTTCGCAGTATTACGTTT
Irr Rev	DNA pulldown/ EMSA	ACAGTACTTCCACTCTCTCTC
Muc2 For	Genotyping	TCCACATTATCACCTTGAC

Muc2 WT Rev	Genotyping	AGGGAATCGGTAGACATC
Muc2 KO Rev	Genotyping	GGATTGGGAAGACAATAG