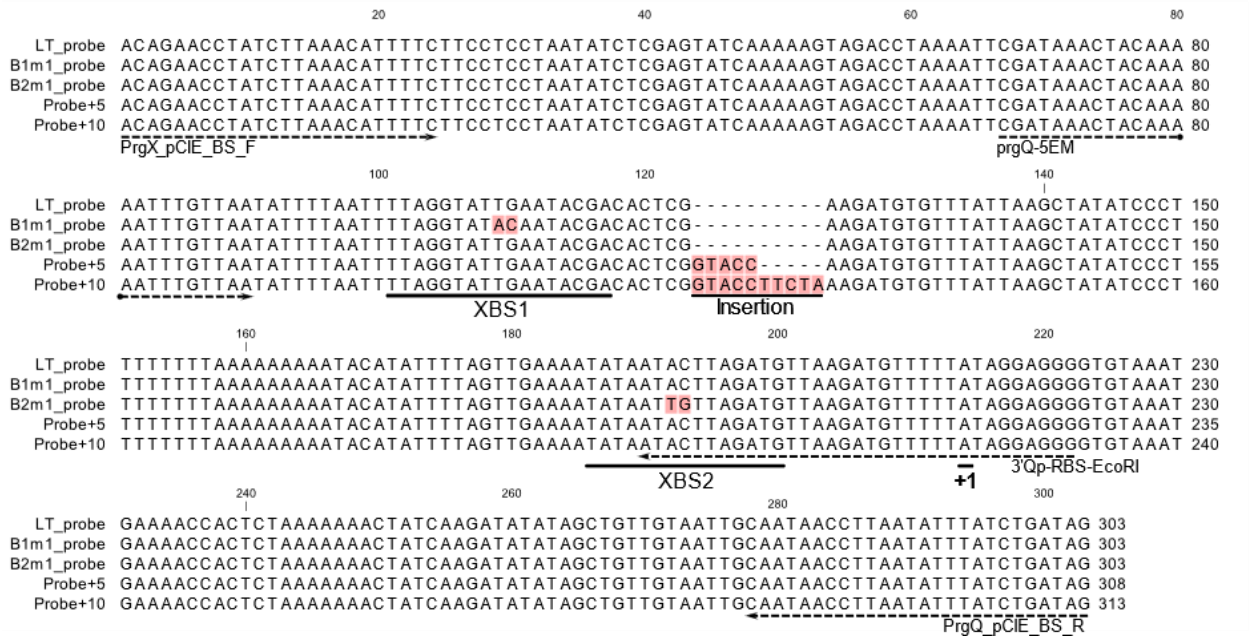
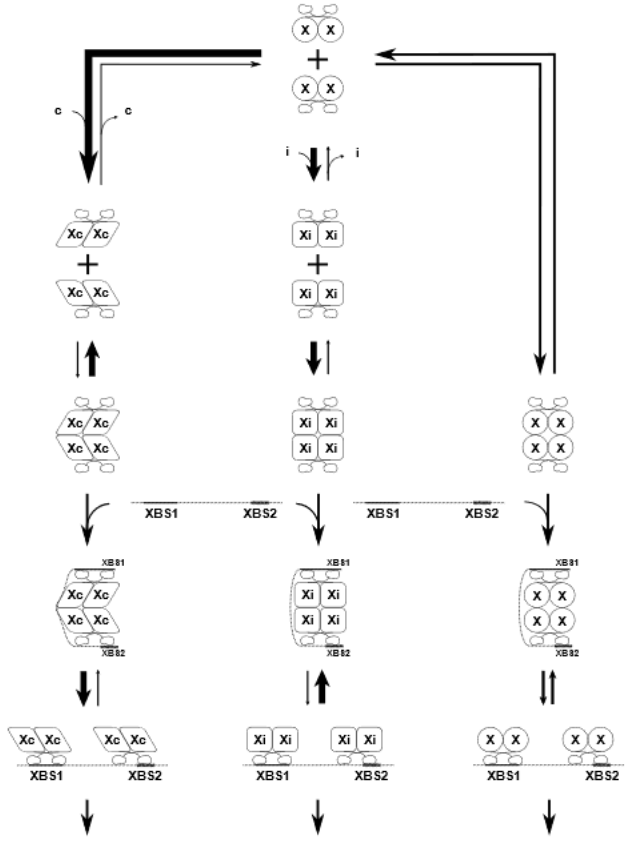


Fig. S1. Sequences of probes used in this study. All probes contain the intergenic region between *prgX* and *prgQ* which is involved in peptide-dependent regulation of *prgQ* transcription by PrgX (1,2,3). The aligned sequences of the probes are shown. The ashed lines below the sequence indicate regions used as templates for PCR primers used to amplify the probe sequences used for EMSA or footprinting assays, asdescribed in the Methods. The red highlight indicates different nucleotides from the WT probe, which has the same sequence as pCF10. The insertion-conntaining probes Probe+5 and Probe+10) have an additional 5 or 10 bp at 124–133 nt. The mutated XBS probes (B1m1 probe and B2m1 probe) have two-nucleotide mutations at 109–110 nt and 202–203 nt, respectively (4). XBS1 and XBS2 regions originally identified by Bae et al. (3) are indicated by horizontal lines below the sequences and the *prgQ* transcription start site is indicated by the bold “+1” above the sequence. Note that XBS2 is located between the -10 and -35 regions relative to the TSS for *prgQ* (5).



Disruption of loop leads to dissociation of XBS2-bound PrgX and induction

Fig. S2. Previous model of PrgX regulation, based on early DNAse footprinting assays examining the binding of Apo-PrgX to pCF10 DNA (3) and on the high-resolution structures of Apo-PrgX, PrgX/***C*** and PrgX/***I*** (2). Apo- PrgX exists as a dimer in solution and these dimers bind XBS1 with high affinity and XBS2 weakly (3). At very high Apo-PrgX concentrations, both XBSs can be occupied (3). All 3 forms of PrgX exist as tetramers in crystals, but the PrgX/***C*** tetramers are distorted with impaired interactions at the interface between dimers, suggesting that PrgX/***C*** tetramers might highly unstable in solution, compared with PrgX/***I*** (2). These predicted differences in oligomerization state in vivo suggested that PrgX/**C** tetramers, whether free or bound to DNA, would not be stable in donor cells, reducing XBS2 occupancy in vivo and resulting in *prgQ* operon induction (2). As illustrated in this figure, Apo-PrgX can bind either ***C*** or ***I*** with high affinity, and bound peptides were predicted to affect oligomerization. All forms of PrgX were predicted to form a looped DNA/PrgX tetramer structure stabilized by cooperative protein/protein and protein/DNA interactions, but higher protein concentrations should be required for Apo-PrgX/DNA tetramers to form. In addition, the tetramer formed by PrgX/***C*** was predicted to be unstable leading to dissociation to dimers. In the case of DNA-bound tetramer complexes, this would open the loop and lead to dissociation from XBS2. Although not shown here, dissociation of unbound tetramers would also inhibit formation of the repressing loop structure. In either situation, the predicted differences in oligomerization state between PrgX/***C*** and PrgX/***I*** would account for the differential activities of the two peptides in regulating *prgQ* expression. More recent data reported by Chen et al. (1) and in the present study suggested modifications of this model as shown in Fig. 1 in the main paper.

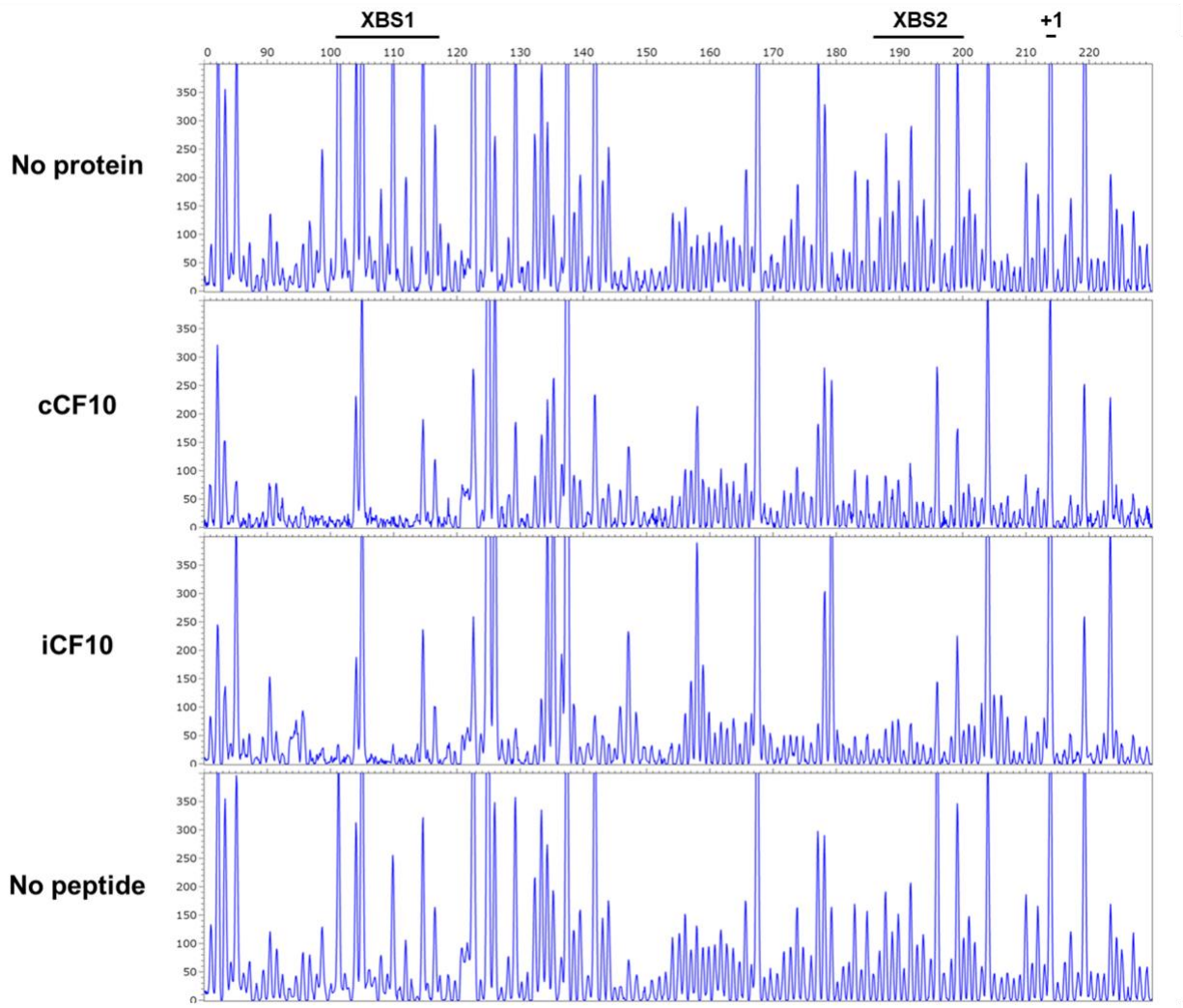

XBS1
XBS2
+1
No protein
cCF10
iCF10
No peptide

Fig. S3. Complete Gene Analyzer elution profile of DNAse-digested WT probe in the Absence (“No protein”) or presence (all other profiles) of various forms of PrgX. The sequence coordinates for the probe are indicated at the top, and the XBS1 and XBS2 lines indicate the regions identified by Bae et al in a previous study using only Apo-PrgX (3). “+1” indicates the transcription start for the *prgQ* operon (5).

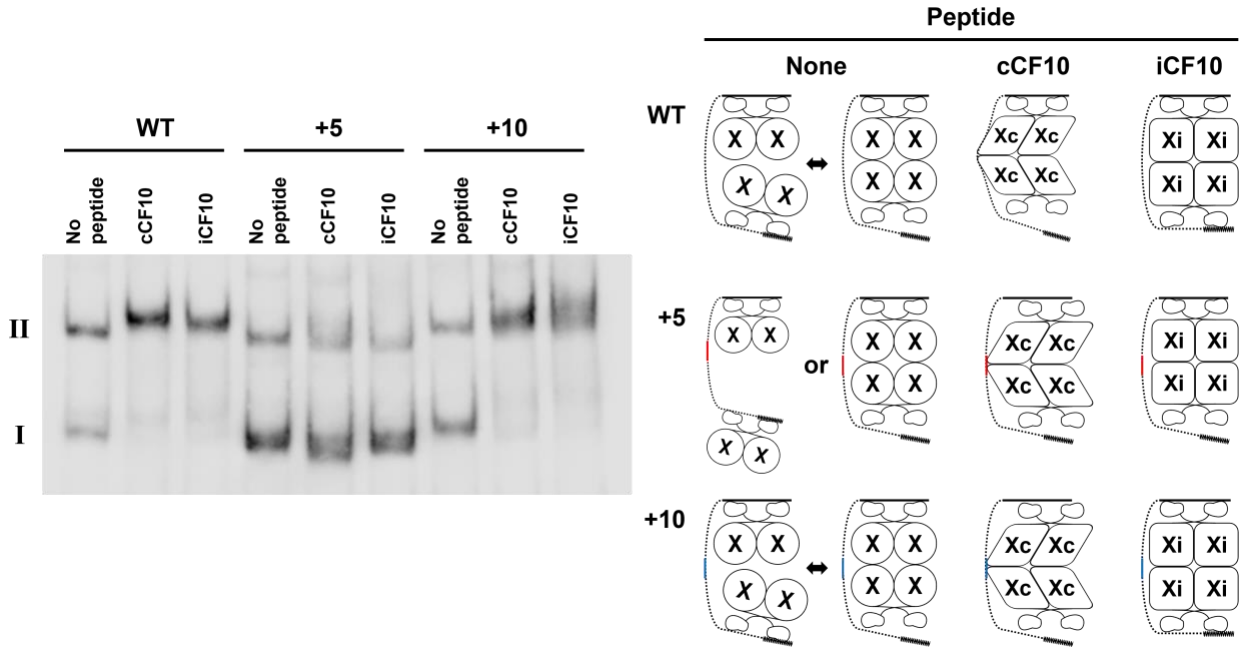


Fig. S4 Comparison of the effect of the insertion between XBSs on PrgX/peptide/DNA complexes by EMSA. The results of EMSA shown in Fig. 3, the shifted bands obtained with the WT probe versus the +5 and +10 probes were compared after extended electrophoresis to amplify small differences in mobility after interaction with 50 nM PrgX/peptide complexes under the same conditions of EMSA shown in Fig. 3 followed by running for 120 min. Unbound bands were not detected because they flowed off the gel. B. Schematic models for PrgX/peptide/DNA complexes.

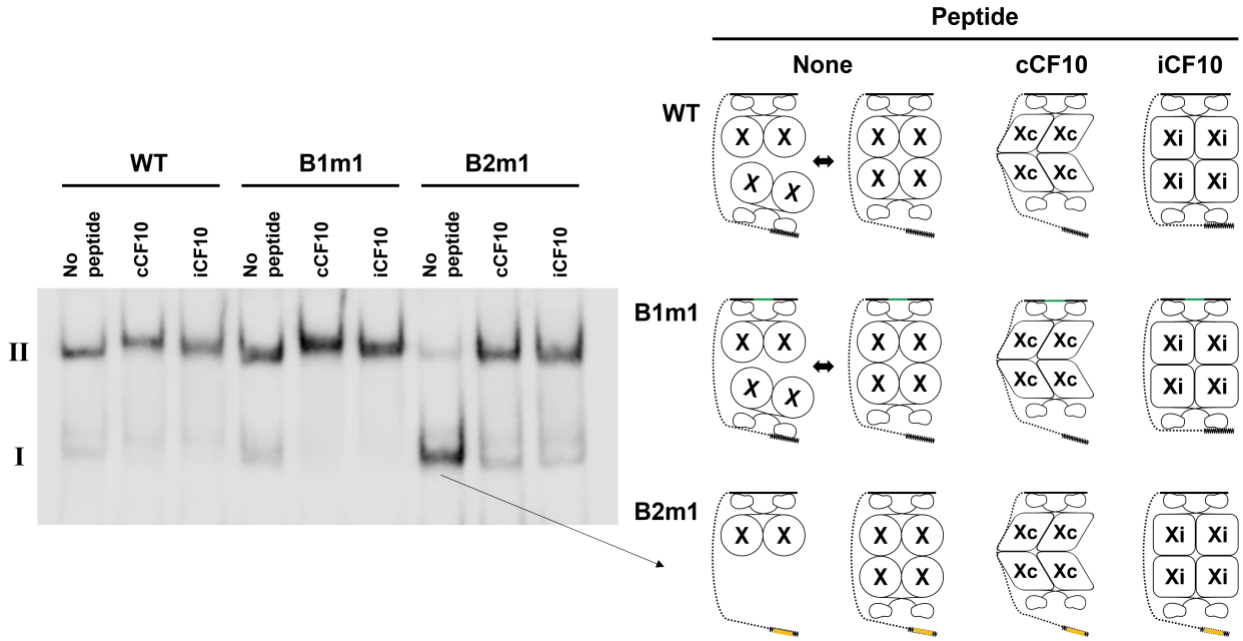


Fig. S5 Comparison of the effect of the XBS mutations on PrgX/peptide/DNA complexes by EMSA. As in Fig. S4, the shifted bands were compared after the probes with XBS mutations and 50nM various PrgX/peptide complexes were reacted under the same conditions of EMSA shown in Fig. 5, followed by running for 120 min. B. Schematic models for PrgX/peptide/DNA complexes.

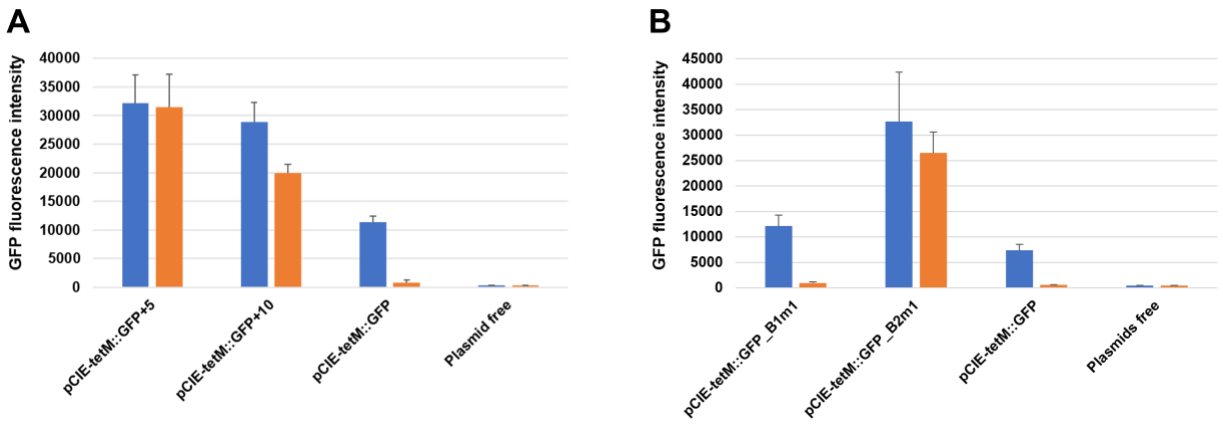


Fig. S6. Biological activity of prgQ regulation in vivo using pCIE-tetM::GFP with insertions or mutated XBSs without additional PrgX source. In Fig.4A and Fig.6A, the effect of the insertions between XBSs or the mutations at XBSs on *prgQ* transcriptional activity by PrgX in vivo. These strains also carried pCF10/G2 to provide wild type levels of PrgX in trans. However, *prgX* gene is also encoded in various pCIE-tetM::GFPs, the transcriptional activity of *prgQ* in strains carrying only pCIE-tetM::GFP without pCF10/G2 was was quantified by measuring GFP expression. A. Strains carrying pCIE-tetM::GFP with insertion between XBSs. B. Strains carrying pCIE-tetM::GFP with XBS mutations. Blue indicates pheromone induced, and orange indicates uninduced. Both sets of strains showed regulation, but a stronger effect was observed in the strains with two plasmids as expected.

References for Supplement

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5) Bensing BA, Meyer BJ, Dunny GM. 1996. Sensitive detection of bacterial transcription initiation sites and differentiation from RNA processing sites in the pheromone-induced plasmid transfer system of *Enterococcus faecalis*. Proc Natl Acad Sci 93:7794–7799.