Supplemental information for:

Translational Independence Between Overlapping Genes for a Restriction Endonuclease and its Transcriptional Regulator

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BMC Molecular Biology

Table S1. Oligonucleotides used in this study

| Oligonucleotide | Sequences (all 5'→3') | Use |
|-----------------|-----------------------|-----|
| PvuLexFor       | TGAGCAGAAATAACATCTTTCATCAGC | Cloning PvuII genes into pLex3B |
| PvuLexRev       | CGGGATCCTCTAATAATTATTTTAGATCTGGGTGACTCATT | |
| PvuTAAFor       | CCCAGATCTGCATAACTACGTCTACGCG | Restoring pvulIC TAA to original location |
| PvuTAARev       | CGCGTAGACGTAGTTATGCAGATCTGGG | QRT-PCR of pvulIC mRNA |
| pvulICFor       | CAAATCCTTTTATCAGCCCGATTAACCC | |
| pvulICRev       | AGGCATTTGGCTTATTCGCTCAATGT | |
| recAFor         | TAACTGAAGCAGTCCAACACGCT | QRT-PCR of recA mRNA |
| recARev         | TTGTTTCCTACACCTTTTCAGCGG | |
| S1TAAFor        | CGAATAGCAAATGCTAATGGTTTCTATTAATAC | Creating stop codons in pvullIR-LacZ fusions upstream of R-initiation site. |
| S1TAARev        | GTATTTGATAGAGGAACATTCATGGCAATTTGCTATTCG | |
| S2TAAFor        | GCCTTAATGTTTCTATATAATAATCTATGATGGAAACAC | |
| S2TAARev        | GTGTTCATCATCATTAGATAATATAGAAACATTTTAAGGC | |
| S3TAAFor        | CTATAACATACATGAGTGTAACACGAAATGAGTCACCCAG | |
| S3TAARev        | CTGGGTGACTCATTTTCATGTTAATCATTAGATTTGTATAG | |
Figure S1. Location of mutations relative to features of C.PvuII. The amino acid sequence of C.PvuII is shown in single-letter code (GenBank AAA96335.1). The DNA-binding helix-turn-helix motif contains the EspI (BlpI) site; fill-in or resection followed by religation duplicates or removes the leucine in the “SLA” sequence. Orange shading indicates the subunit interface, from comparison to the crystal structure for an orthologous C protein [1]. This interface spans the ClaI site used to generate frameshift mutations. The two in-frame deletions of predicted RNA hairpin elements (see Fig. 7A) are indicated as Δ1 and Δ2. The position of the alternate reading frame pvuII R initiation codon is indicated.
Figure S2. Effects of distance between termination and initiation codons on translational reinitiation. This is a replotting of data from [2]. The lacZ gene was introduced into E. coli with varied spacing between the lacZ initiation codon and the terminator for the upstream ORF.
Figure S3. Putative RNA hairpins upstream of REase gene initiation codon in selected C-dependent R-M systems. C-dependent R-M systems, from among those shown in Fig. 2, were analyzed for potential hairpin structures upstream of the REase gene initiation codon. The 40 nt upstream of each initiator were submitted to MFOLD [3], and the most stable structure returned in each case is shown. The number in parentheses is the predicted ΔG of folding, in kcal/mol. The Esp1396I structure may be further stabilized by its GNRA loop [4]. The initiator codon and predicted RBS are highlighted in green. Secondary structures are shown for comparison, whether or not they are likely to be present a significant fraction of the time.
References

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4. Heus HA, Pardi A: Structural features that give rise to the unusual stability of RNA hairpins containing GNRA loops. *Science* 1991, 253(5016):191-194.