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Translational frameshift sites within bacteriophage λ genes *rexA* and *cI*°

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Phage λ's *cI-rexA-rexB* operon displays an intriguing example of regulation by an unexplained mechanism of polarity. We have identified three potential −1 translational frameshift sites and present a model for translational frameshift suppression by lambda's CI repressor as a mechanism of regulating operon polarity, implying an additional role for CI self-regulation.

In prokaryotes translation occurs concurrently with transcription. Mutations that result in abortive translation (mutation to a stop codon) frequently also cause premature termination of transcription *via* a rho-dependent mechanism [1]. In operons, such mutations have a polar effect on the expression of genes transcribed downstream. We searched for a

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Abbreviations: A, adenosine; aa, amino acids; anti-db, sequence on 16S rRNA complementary to db on message; bp, base pairs; db, downstream box on mRNA complementary to 16S rRNA; G, guanosine; genes: *cI* [encodes an autoregulatory DNA binding protein (repressor) that stimulates its own transcription from p_M, while in turn repressing transcription of λ lytic genes], *rexA* and *rexB* (genes downstream from *cI* whose expression is required for Rex exclusion phenotype of λ lysogenic cell); N, any nucleoside; *p* promoter; promoters: p_M (promoter for expression of λ *cI-rexA-rexB* operon from prophage within lysogen) and p_{Lit} (independent promoter for *rexB*); SD, Shine-Delgarno sequence; Ts, temperature sensitive.

rationale to account for the transcriptional polarity seen within the bacteriophage lambda (λ) p_M-*cI-rexA-rexB* operon when CI repressor is thermally inactivated under conditions allowing efficient *cI* translation ([2], unpublished citations in [3], Hayes, Bull & Slavcev, unpublished data). Conditional (Ts, temperature sensitive) expression of the Rex exclusion phenotype, during conditions where the *cI-rexA-rexB* operon is transcribed and the CI857Ts repressor is thermally inactivated has also been described [3]. The same operon shows no transcriptional polarity when the CI repressor is fully active [4, 5]. Gene *cI* encodes the λ repressor protein CI, an autoregulatory DNA binding protein that stimulates its own transcription from the promoter p_M, while repressing transcription of λ lytic genes. Genes *rexA* and *rexB* are downstream from *cI* and are required for the Rex exclusion phenotype in which λ lysogenic cells are immune to infection by certain infecting phage. Certain mutants of λ are sensitive to Rex exclusion [6, 7], suggesting that λ may itself be sensitive to Rex exclusion if *rexA* and *rexB* are inappropriately overexpressed. The precise mechanism of Rex exclusion remains to be elucidated, but RexB is proposed to be a regulated pore that is opened to depolarize the cytoplasmic membrane [7]. p_M Is the maintenance promoter for expression of the *cI-rexA-rexB* operon from the λ prophage DNA within a lysogen.

A rho-dependent termination site has been described within the p_M transcript [2]. However, this terminator is active only in the absence of *cI* translation [2] and its presence alone cannot account for the transcriptional polarity seen when CI repressor is thermally inactivated under conditions allowing efficient *cI* translation. We wondered if translational frameshifting might be occurring in the operon. In *Escherichia coli* at least two genes have been characterized that use translational frameshifting to produce either two different products from the same gene [8], or to regulate expression of a gene product [9]. In the *dnaX* gene of *E. coli* the presence of a heptameric sequence AAAAAAG within codons NNA-AAA-AAG, leads to efficient −1 frameshifting due to slippage of $tRNA^{lys}$ [8]. We examined the 2136 bp *cI-rexA-rexB* operon and found it encodes two A-AAA-AAG sequences within the open reading frame for *cI*, and another such sequence within the open reading frame for gene *rexA* (Fig. 1A). The AAAAAAG sequence is expected to occur randomly once per 49,152 translated base pairs (bp) in the A-AAA-AAG reading frame (i.e., once per 4^7 bp X three possible reading frames per strand). The probability of three such sites occuring by random chance within the 2136 bp operon is extremely low ($P < 0.0001$). The two sites in *cI* are within codons 3–5 and 24–26 at the 5′ end of the *cI* mRNA. Translational stop codons are present in the −1 reading frame immediately following each sequence in *cI* and *rexA* (Fig. 1A).

In *dnaX* the efficiency of frameshifting allowed the −1 frameshift product to be produced in nearly equivalent amounts compared to the unshifted product. Translational frameshift systems can include auxiliary signals in the mRNA that facilitate ribosome pausing and enhance frameshifting at "slippery codon" sequences [10, 11]. The −1 translational frameshifting event in *dnaX* is stimulated by the presence of a stem-loop structure downstream of the AAAAAAG sequence in the mRNA, believed to increase ribosome pausing [8]. However, −1 frameshifting did occur on mRNA's lacking the stem-loop structure. They postulated that stable interactions between the translating ribosome and a nucleotide sequence upstream of a frameshift consensus site could abet ribosome pausing, and increase translational frameshifting events.

There is some evidence that the A-AAA-AAG sites in the *cI* gene are potential ribosome pause sites. Overlapping sequences were identified by Balakin *et al.* [12] as 30S binding sites in the p_M-initiated-*cI*-message using a

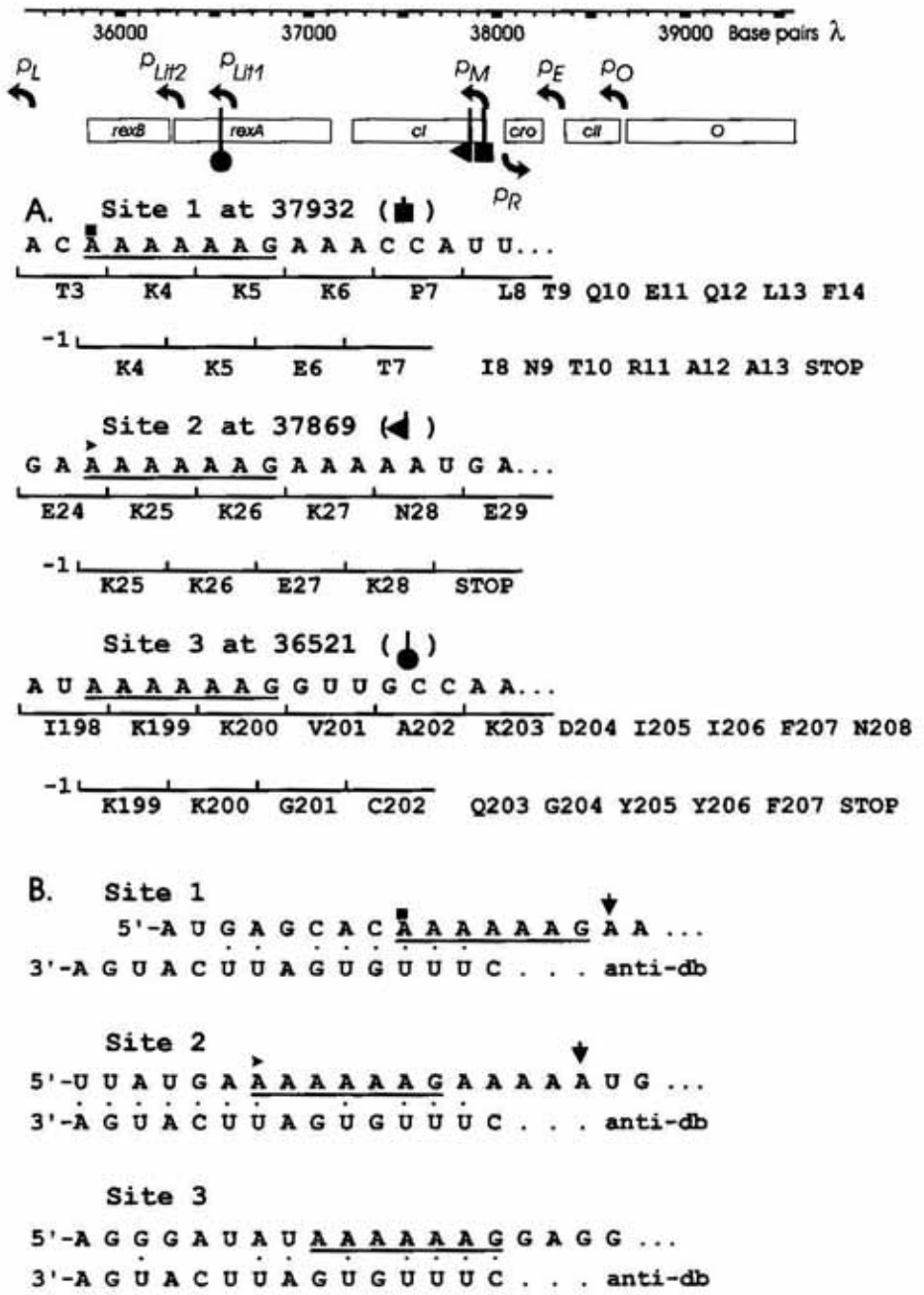

Figure 1. ***Inserted map*: The *cI-rexA-rexB* operon is expressed from a noninduced prophage from promoter p_M.**

Two putative downstream promoters for *rexB* are drawn: p_{Lit2} is estimated from *in vitro* studies [17] and sequence analogy [18]; p_{Lit1} was identified from an induced prophage and remapped using sequence information for markers and original data [16, 19]. *Part* **A.** Coding sequences within the *cI-rexA-rexB* operon with the slippery heptamaric sequence A-AAA-AAG were found within reading frames for genes *cI* and *rexA*. Two sites found within *cI* were very near to the 5′ end of the 710 bp gene. The slippery shift site 1 can produce a -1 translational frameshift at codons K4–K5 of *cI*, which will terminate 8 amino-acid residues downstream. Shift site 2 occurs at codons K25–K26 of *cI*, terminating translation 2 amino-acid residues downstream. Site 3 within *rexA* occurs at K199–K200 with termination 7 amino-acid residues downstream. *Part* **B.** Regions of complementarity (indicated by dots) between the anti-db sequence [15] of 16S rRNA and *cI* mRNA are shown relative to the potential -1 translational frameshift sites (underlined). The *cI* mRNA sequences that were bound by 30S ribosomal subunits in toe print assays of Balakin *et al.* [12] include db (see text) and shift sites 1 and 2 (site 3 remains untested). The limit of rightward binding is indicated by the arrow located 3 to 4 nucleotides to the right of the anti-db sequence. Anti-db homology also occurs upstream of site 3 at 36500 (not shown).

toe-print assay. The p_M-initiated *cI* transcript is unusual in that it begins with the first A of the AUG translation initiation codon. The message therefore lacks any 5′ leader sequence that would normally contain the Shine-Delgarno box ribosome recognition sequence (SD). Some evidence suggests that the p_M initiated *cI* transcripts are translated by a subset of ribosomes deficient in ribosomal protein S2 [13, 14]. Sequences important for initiation of translation of p_M transcripts are downstream and were termed *d*ownstream *b*ox (db, sequence on mRNA complementary to 16S rRNA). The db bound to the bases 1470–1484 of 16S rRNA and were termed the antidownstream box (anti-db) [15]. We examined the *cI-rexA* sequences around the A-AA-A-AAG sites for potential complementarity to the anti-db sequence of 16S rRNA with the rational that the presence of db at internal sites would cause strong ribosome binding to facilitate pausing. Sequence intervals with homology to the anti-db sequence on 16S rRNA were found to precede or cover each of the three potential frameshift sites within *cI* and *rexA* (Fig. 1B). Frameshift site 2 and the db sequence are very similar. Thus the putative frameshift sites are near the vicinity of known ribosome binding sites [12] which are likely binding 16S rRNA *via* db/anti-db interactions. It seems probable that an interaction between db and anti-db sequences would influence translational frameshifting by serving as ribosome pause sites.

This information provides a possible explanation for the transcriptional polarity and loss of the Rex exclusion phenotype observed in the *cI-rexA-rexB* operon when transcribed from p_E (i.e. the establishment promoter for *cI-rexA-rexB* operon activated by λ *cII* gene product) following inactivation of CI(Ts) repressor by temperature shift [3]. It is known that the CI repressor activates transcription of *cI-rexA-rexB* from p_M but not from p_E. We propose that the repressor acts to prevent frameshifting at sites 1–3 by suppressing ribosome pausing at db sequences overlapping the frameshift sites *via* CI interaction with ribosomal proteins. By stimulating its own translation, the CI repressor would also be required for efficient expression of downstream genes in the p_E *-cI-rexA-rexB* operon.

WHAT ARE THE RAMIFICATIONS FOR λ's LIFESTYLE?

Prophage induction. Loss of p_M stimulation and the activation of a rho-dependent termination event in *cI* contribute to loss of CI repressor during the switch to lytic growth. In addition to the enhanced autoregulatory aspects of *cI* expression, these events would both greatly facilitate the rapid turn-off of the Rex exclusion system, as follows. Repressor inactivation would result in a rapid reduction in downstream *rexA* expression. In contrast, some level of *rexB* expression from the downstream promoter p_{Lit} (an independent promoter for *rexB*, Fig. 1) [16, 17, 18] is constitutive [16, 18]. Recently, Hayes *et al.* [19] distinguished two p_{Lit} promoters separated by about 300 bp. The promoter p_{Lit2} was constitutive for a low level of *rexB* expression, and the upstream p_{Lit1} promoter allowed for inducible expression of *rexB*. The *rexB* transcription from the p_{Lit} promoters would serve to increase the RexB:RexA ratio upon inactivation of the CI repressor. The increased RexB:RexA ratio will suppress Rex exclusion [7] and, we propose, permit a derepressed λ prophage to escape from its own Rex exclusion system.

Lysogenic decision. During establishment of the lysogenic state, fine-tuning of RexA expression would facilitate a delay in the expression of Rex exclusion until the lysis/lysogeny decision has been made and CI repressor accumulates.

DO KNOWN *cI* MUTATIONS SUPPORT THE MODEL?

Nasi *et al.* [20] isolated pleiotropic mutants of λ that could establish stable lysogeny, and yet confer a Rex⁻ phenotype. These mutations (called C*) mapped to the 5′-end of gene *cI* and were able to complement *rexB* mutations. Thus, the C* mutants were deficient in *rexA* expression, yet they mapped to the amino end of *cI*, between markers spi275 in *cI* [21] and spi274 within o_{R2} [21, 22]. The A-AAA-AAG sites 1 and 2 fall within this interval. The sequenced *cI* mutations [23] arising within the frameshift consensus site 1 (*cI*57-1, *cI*S57, *cI*SP62, *cI*UA60) or site 2 (*cI*ET28) either change the amino-acid composition of *cI*, or the downstream reading frame (*cI*UA60 is a single bp insertion). Deciphering whether a *cI* mutation affects frameshifting is made difficult because its phenotype would be expected to be similar to that of *cI* mutations reducing o_R binding, or perturbing the interaction of RNA polymerase with p_M. Specific site-directed mutational analysis will be required to directly test this model.

The proposed mechanism adds yet another level of potential regulation in the complex decision process of lytic *versus* lysogenic pathways. The potential regulation of these translational frameshifts may yield additional insights into the mechanics of ribosome fidelity.

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