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Resistance of λ *c*I Translation to Antibiotics That Inhibit Translation Initiation

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The lambda *c*I lysogenic transcript is unusual in having no leader. Expression of a *c*I-*lacZ* protein fusion was relatively resistant to kasugamycin and pactamycin, which inhibit translation initiation on transcripts with leaders. Our data imply that there are distinct differences in translation initiation between the two classes of transcripts.

Escherichia coli mRNAs generally have a 5′ leader which includes a purine-rich sequence, the Shine-Dalgarno sequence (SD) (for exceptions, see references 1, 10, 17, and 25). The SD is located 4 to 15 nucleotides upstream of the initiation codon and has complementarity to the 16S rRNA (5). The SD facilitates binding of the 30S ribosomal subunit to the mRNA transcript by base pairing with the 16S rRNA (7, 9).

It is likely that sequences other than the SD and the initiation codon make relevant ribosomal contacts that are involved in translation initiation, as suggested by RNase protection experiments (21). Some *E. coli* genes require a specific sequence within the coding region known as the downstream box (db) for their efficient expression (3, 8, 14, 20). The db, like the SD, has complementarity to the 16S rRNA (20). It has been shown that efficient expression of λ *c*I requires the db (19).

Transcription of *c*I can begin at one of two promoters, pE or pM (18). pE is expressed early after infection, and the mRNA it produces contains a leader sequence. pM is responsible for the maintenance of repressor levels in a lysogen. Unlike pE transcripts, pM transcripts start with the A of the AUG initiation codon and therefore lack a 5′ leader and an SD (17, 25).

It has been shown that mutations in *rpsB* that reduce the levels of ribosomal protein S2 stimulate translation of the *c*I lysogenic transcript, presumably through more efficient use of the *c*I db (19). Loss of S2 increases the affinity of ribosomes for the *c*I transcript and decreases ribosome binding to leadered mRNA. Interestingly, alterations in S2 confer resistance to the antibiotic kasugamycin (16, 27), an inhibitor of translation initiation (15, 23). Furthermore, photoaffinity labeling studies show that S2 associates with the antibiotic pactamycin (24), which also inhibits translation initiation (2, 23). If kasugamycin and pactamycin inactivate S2 or ribosomes containing S2, *c*I translation may be resistant to inhibition by these antibiotics.

We tested the effects of kasugamycin and pactamycin on *c*I expression in three *E. coli* strains. CS282/pλ*c*I(14)P carries a *c*I-*lacZ* protein fusion which is expressed from the pM promoter and includes the *c*I db (19). CS282/pλ*c*I(14)O expresses a *c*I-*lacZ* operon fusion from pM: *lacZ* carries its own translational signals, including an SD (19). N7762 expresses the *lac* operon from the L8UV5 promoter. We also tested the effects of chloramphenicol and tetracycline, which inhibit translation elongation (4). Because pactamycin at high concentrations also inhibits elongation (11, 22), we used all antibiotics at concentrations that gave approximately 50 to 60% growth inhibition.

Cells were grown in Luria broth (LB), or LB plus ampicillin (50 μg/ml) in the case of plasmid-bearing cells, at 37°C until mid-log phase (optical density at 650 nm = 0.3 to 0.5). Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to N7762 to induce expression of *lacZ*. To ensure steady-state expression of *lacZ* prior to antibiotic addition, cultures were maintained in logarithmic growth for 4 to 5 h by repeated dilution with fresh media. Antibiotics were added at 0 min, and cultures were assayed for β-galactosidase activity every 30 min, up to 90 min (12).

The results of these experiments are shown in Fig. 1. In the absence of antibiotics, the specific activity of β-galactosidase remained unchanged in each strain. The expression of *lacZ* was particularly sensitive to all the antibiotics tested. The specific activity of β-galactosidase expressed from the *c*I-*lacZ* operon fusion or from the chromosomal *lacZ* was reduced significantly during the time course of the experiment (Fig. 1b and c). The expression of the *c*I-*lacZ* protein fusion responded quite differently to the antibiotics. The specific activity of β-galactosidase from the protein fusion increased significantly after addition of kasugamycin and pactamycin and was unchanged after treatment with chloramphenicol and tetracycline (Fig. 1a). These results indicate that the translation of the leaderless *c*I transcript is less sensitive to kasugamycin and pactamycin than translation of other cellular transcripts. The translation of *lacZ*, on the other hand, is more sensitive to these antibiotics, as well as to chloramphenicol and tetracycline.

Kasugamycin and pactamycin may enhance translation of *c*I by inhibiting S2 or ribosomes that contain S2. In this fashion, these antibiotics would reproduce the phenotype of *rpsB* mutants that overexpress *c*I (19). Alternatively, kasugamycin and pactamycin may directly block the initiation site on the ribosome (26). Nucleotides in 16S RNA that are protected by kasugamycin and pactamycin are also protected by initiator tRNA (13, 26). Furthermore, alterations in methylation of adenosine residues adjacent to the 16S RNA anti-SD confer kasugamycin resistance (6). According to this model, kasugamycin and pactamycin may block interactions between the SD and the 16S RNA without interfering with contacts made via the db.

Although tetracycline and chloramphenicol did not significantly alter the relative expression of β-galactosidase from the *c*I-*lacZ* protein fusion in these experiments, higher concentra-

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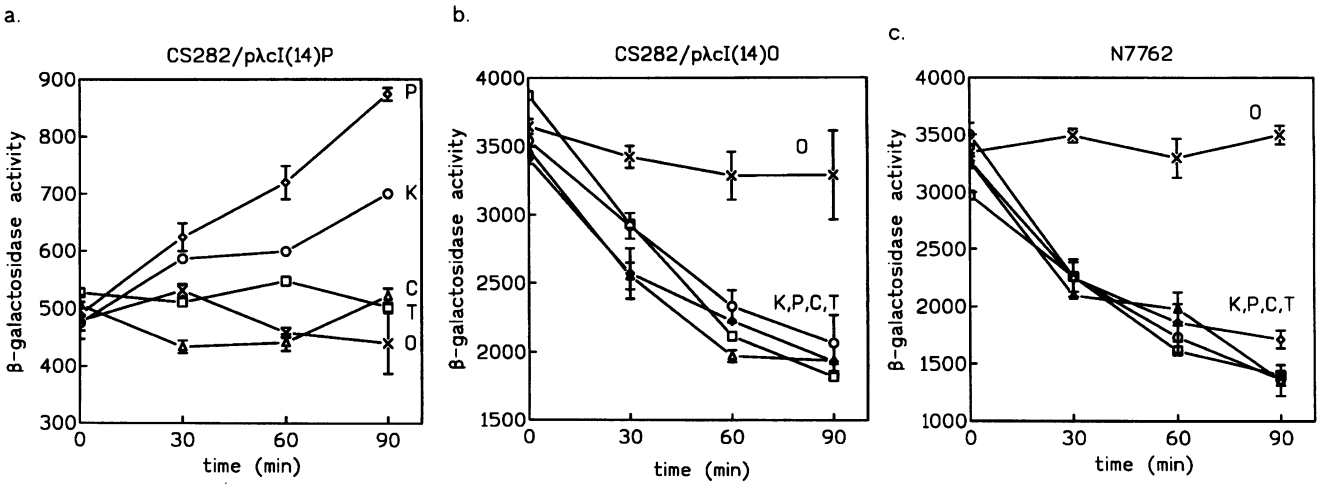

FIG. 1. Effect of antibiotics on β-galactosidase-specific activity of λ *c*I protein and operon fusions and of *lac*L8UV5. (a) Strain CS282 (W3102 *str galK2*) carrying pλ*c*I(14)P, a plasmid-borne *cI-lacZ* gene fusion, driven by pM, that joins the first 14 codons of *c*I to the ninth codon of *lacZ*. (b) Strain CS282 carrying pλ*c*I(14)O, a plasmid-borne operon fusion, driven by pM, that expresses full-length *lacZ* with its own translation initiation signals. (c) Strain N7762 (W3102 *str galK2 lac*L8UV5) in the presence of 1 mM IPTG. O, no antibiotic; C, chloramphenicol, 2 μg/ml; K, kasugamycin, 200 μg/ml; P, pactamycin, 5 μg/ml; T, tetracycline, 0.2 μg/ml. Data are average values of two assays. Absence of error bars indicates that assay values were within 10 units.

tions of these antibiotics were inhibitory (data not shown). Translation initiation of *c*I is inefficient (19) and likely remains rate limiting during treatment with low concentrations of elongation inhibitors.

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