Erythromycin Induces Expression of the Chloramphenicol Acetyltransferase Gene *cat-86*

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The plasmid gene *cat-86* specifies chloramphenicol-inducible chloramphenicol acetyltransferase in *Bacillus subtilis*. This gene, like the erythromycin-inducible *erm* genes, is regulated by translational attenuation. Here we show that *cat-86* is also inducibly regulated by erythromycin. *cat-86* does not confer resistance to erythromycin.

The regulated expression of the chloramphenicol acetyltransferase gene *cat-86* is due to translational attenuation (7). The gene is continuously transcribed, but transcripts are not (or are very poorly) translated because the *cat-86* ribosome-binding site is sequestered in a stable stem-loop structure. Chloramphenicol induces *cat-86* by stalling a ribosome at a precise location in the leader region of *cat-86* transcripts; this region is located 5' to the stem-loop structure (Fig. 1). The stalled ribosome is thought to destabilize the stem-loop, permitting translation of the *cat-86* coding sequence. Other inducible *cat* genes identified in both *Bacillus* and *Staphylococcus* spp. are preceded by similar regulatory sequences and are likely regulated by a comparable mechanism (4, 7).

cat-86 confers chloramphenicol resistance on host bacteria; the *cat-86* product chloramphenicol acetyltransferase acetylates chloramphenicol, thus inactivating the antibiotic activity of the drug. Therefore, the inducer for *cat-86* is also the substrate for the corresponding enzyme. *cat-86* is also induced by the nucleoside antibiotic ampicillin (6). However, the *cat-86* product does not confer resistance to ampicillin. In contrast, *cat* genes identified on the small *Staphylococcus aureus* plasmids pC221 and pC194 are chloramphenicol inducible but fail to respond to ampicillin as an inducer. Here we show that *cat-86* is induced by the antibiotic erythromycin (Fig. 2), whereas *cat* genes on plasmids pC194 and pC221 are not detectably induced by this antibiotic.

Chloramphenicol induction of *cat-86* transcripts depends on an intact regulatory sequence, which extends from ribosome-binding site 2 through the beginning of the *cat-86* coding sequence (2). Deletions in the parent plasmid pPL703 from the linker to a site immediately 5' to ribosome-binding site 2 (as in the *cat-86*Δ60 mutant; Fig. 1) permitted induction by chloramphenicol and also by erythromycin (Table 1). A deletion that extended 25 base pairs further (*cat-86*Δ85; Fig. 1) removed ribosome-binding site 2, and this deletion prevented induction by chloramphenicol. Similarly *cat-86*Δ85 was not erythromycin inducible (Table 1).

Ochre mutations at *cat-86* leader codons 5 and 6 have dramatically different effects on induction (1). The ochre mutation at leader codon 5 prevented induction by both chloramphenicol and erythromycin (Table 1). The ochre mutation at leader codon 6 resulted in a high basal level of expression but permitted induction by chloramphenicol and also by erythromycin (Table 1).

cat-86 is not induced by lincomycin or by tetracycline. We

previously believed that erythromycin was not capable of induction of *cat-86*, because the antibiotic failed to yield a positive result in an induction plate test (6). This test clearly gave a false-negative in the case of erythromycin, although in general we found that the plate test tended to give false-positives. The discovery that erythromycin induced *cat-86* was the unexpected result of a control. Expression of leader codons 2 through 5 of *cat-86* (the *crb-86* box) retards the doubling time of *Bacillus subtilis* cells grown in the presence of low levels of those antibiotics that induce the gene (E. J. Rogers, N. P. Ambulos, Jr., and P. S. Lovett, submitted for publication). This is thought to be due to transient trapping of ribosomes on the *crb-86* box caused by stalling and base pair formation between *crb* and 16S rRNA. Lincomycin, which does not induce *cat-86*, did not selectively slow the growth of *crb*-containing cells, whereas chloramphenicol, fluorothiamphenicol, and ampicillin did. Erythromycin also slowed the growth of cells expressing *crb*, which led us to test erythromycin as an inducer of *cat-86*.

Erythromycin is the known inducer of another gene family, the *erm* genes, which are also regulated by translational attenuation (5). A recent publication described the fusion of

FIG. 1. Structure of the regulatory region of *cat-86* transcripts. The UUG codon to the right is the beginning of the *cat-86* coding sequence. The Δ60 and Δ85 deletion end points are indicated by arrows. A and P designate the codons occupied by the aminoacyl and peptidyl sites, respectively, of a chloramphenicol-stalled ribosome (7). RBS, Ribosome-binding site.

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FIG. 2. Induction of *cat-86* by erythromycin. Plasmid pPL703-Spac contains the *cat-86* gene (7). The plasmid was transformed into *B. subtilis* BR151. (Left) Dose-response curve obtained by incubating BR151(pPL703-Spac) in Penassay broth with various concentrations of erythromycin for 2 h. Cells were lysed and chloramphenicol acetyltransferase (CAT) and total protein were determined (3, 9). Specific activity is reported as micromoles of chloramphenicol acetylated per minute per milligram of protein at 25°C. (Right) Time course of induction, determined by incubating BR151(pPL703-Spac) with 0.05 μg of erythromycin per ml at 37°C. Samples were periodically removed for assay of chloramphenicol acetyltransferase.

TABLE 1. Response of mutant derivatives of *cat-86* to induction by erythromycin^a

<i>cat-86</i> gene	CAT sp act with the following inducer:		
	None	Erythromycin	Chloramphenicol
Wild type	0.45	5.04	4.8
Δ60	0.30	1.59	1.13
Δ85	0.06	0.09	0.09
TAA-5	0.12	0.17	0.11
TAA-6	1.47	4.31	3.10

^a *cat-86*Δ60 and *cat-86* Δ85 contain deletions into the 5' end of the regulatory leader (2). Deletion endpoints are shown in Fig. 1. *cat-86* TAA-5 and *cat-86* TAA-6 contain ochre mutations at leader codons 5 and 6, respectively. BR151 carrying derivatives of pPL703-Spac that contained the various *cat-86* leader mutations was grown at 37°C with no inducer or with 0.05 μg of erythromycin or 2 μg of chloramphenicol per ml for 2 h, at which time chloramphenicol acetyltransferase (CAT) was assayed. Specific activity is reported as micromoles of chloramphenicol acetylated per minute per milligram of protein.

the *ermC* leader to the 5' end of the *cat-86* leader, and this reportedly caused *cat-86* expression to become erythromycin inducible (8). This interpretation will require reevaluation based on our current findings.

Presently we do not know whether erythromycin induction depends on the same sequences in the *cat-86* leader that are required for chloramphenicol induction. However, the relative simplicity of the *cat-86* regulatory sequence may aid in the clarification of the action of erythromycin as an inducer of a gene regulated by translational attenuation.

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