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Chloramphenicol-Erythromycin Resistance Mutations in a 23S rRNA Gene of *Escherichia coli*

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Two chloramphenicol resistance mutations were isolated in an *Escherichia coli* rRNA operon (*rrnH*) located on a multicopy plasmid. Both mutations also confer resistance to 14-atom lactone ring macrolide antibiotics, but they do not confer resistance to 16-atom lactone ring macrolide antibiotics or other inhibitors of the large ribosomal subunit. Classic genetic and recombinant DNA methods were used to map the two mutations to 154-base-pair regions of the 23S RNA genes. DNA sequencing of these regions revealed that chloramphenicol-erythromycin resistance results from a guanine-to-adenine transition at position 2057 of the 23S RNA genes of both independently isolated mutants. These mutations affect a region of 23S RNA strongly implicated in peptidyl transfer and known to interact with a variety of peptidyl transferase inhibitors.

Dominant or codominant antibiotic resistance mutations in *Escherichia coli* rRNA genes can be isolated when the contribution of altered RNA to ribosomes is increased by having the mutant *rrn* operon on a multicopy plasmid. This approach has allowed the previous isolation of a spectinomycin resistance mutation that alters position 1192 of 16S RNA (20, 25) and a mutation altering position 2058 of 23S RNA which confers resistance to the lincosamide, streptogramin type B, and 14- and 16-atom lactone ring macrolide antibiotics (24, 25). This paper describes the isolation of two identical mutations that alter position 2057 of 23S RNA and confer resistance to chloramphenicol and the 14-atom lactone ring macrolide antibiotics. The two mutations do not cause resistance to 16-atom lactone ring macrolide antibiotics or other inhibitors of the large ribosomal subunit.

A number of bacteriostatic antibiotics, including chloramphenicol and the macrolide, lincosamide, and streptogramin type B antibiotics, inhibit the peptidyl transferase region of the 50S ribosomal subunit (reviewed in reference 12). In this paper we define the peptidyl transferase region as the peptidyl transferase active site and whatever channels or binding sites are needed for the substrates to be recognized at the peptidyl transferase active site. Since all elements of the peptidyl transferase region are probably located in a small region of the 50S ribosome subunit, are structurally ill defined, and possibly undergo concerted conformational changes, it is not surprising that peptidyl transferase region inhibitors generally inhibit more than one function of the peptidyl transferase region (reviewed in reference 12). The multiple functions of the peptidyl transferase region, coupled with the use of widely divergent ribosomal and subribosomal assay systems, have resulted in confusing and sometimes contradictory information about the mode of action of peptidyl transferase region inhibitors (judiciously reviewed in reference 12).

Despite the problems associated with interpretation of the data obtained by using peptidyl transferase inhibitors, it has long been clear that different peptidyl transferase inhibitors behave differently even in a single assay system. For example, chloramphenicol, 16-atom lactone ring macrolide antibiotics, and lincosamide antibiotics inhibit the peptidyl transferase active site in most assay systems (7, 12, 13, 17, 19, 21), whereas streptogramin type B antibiotics and 14-atom lactone ring macrolide antibiotics usually do not (12, 19, 21). In some assay systems, erythromycin (a 14-atom lactone ring macrolide antibiotic) stimulates peptidyl transfer (19, 21). Certain 16-atom lactone ring macrolide antibiotics (of the niddamycin and spiramycin subgroups) also appear to inhibit substrate binding or recognition (4, 5, 23), whereas erythromycin, chloramphenicol, and the streptogramin type B antibiotics do not (4, 5, 12, 23, 33). Erythromycin may primarily affect translocation (12, 15, 31), whereas streptogramin type B antibiotics have no clear effect on any single well-defined function of the peptidyl transferase region (reviewed in reference 12).

The ribosome inhibition studies described above illustrate similarities and differences within and between the structurally related classes of peptidyl transferase region inhibitors. Mutations in ribosomal components can also be useful in illustrating the overlaps in binding sites or modes of action of peptidyl transferase inhibitors. For example, an overlap of binding sites or functional sites is illustrated by a mutation (24) or methylation (26) of position 2058 of bacterial 23S RNA which confers resistance to 14- and 16-atom lactone ring macrolide, lincosamide, and streptogramin type B antibiotics (MLS antibiotics) but do not confer resistance to chloramphenicol. Nonidentity of binding or functional sites for chloramphenicol and MLS antibiotics is also illustrated by mitochondrial rRNA mutations that confer resistance to erythromycin but which have not been reported to cause resistance to chloramphenicol (28) and by other mitochondrial rRNA mutations that cause resistance to chloramphenicol but which have not been reported to cause resistance to erythromycin (2, 9, 16, 27). In this paper we describe two mutations which differentiate between the 14- and 16-atom lactone ring macrolide antibiotics and establish an overlap between the binding sites or modes of action of chloramphenicol and 14-atom lactone ring macrolide antibiotics.

MATERIALS AND METHODS

Bacterial strains and plasmids. All starting bacterial strains and plasmids have been previously described (20, 24, 25). Details of the plasmid structures are given in Fig. 1. Plasmid pLC7-21 confers immunity to colicin E1 and is transmissible by plasmid F. Plasmid pLC7-21 replicates from the ColE1 origin and is compatible with plasmid F and the amplifiable

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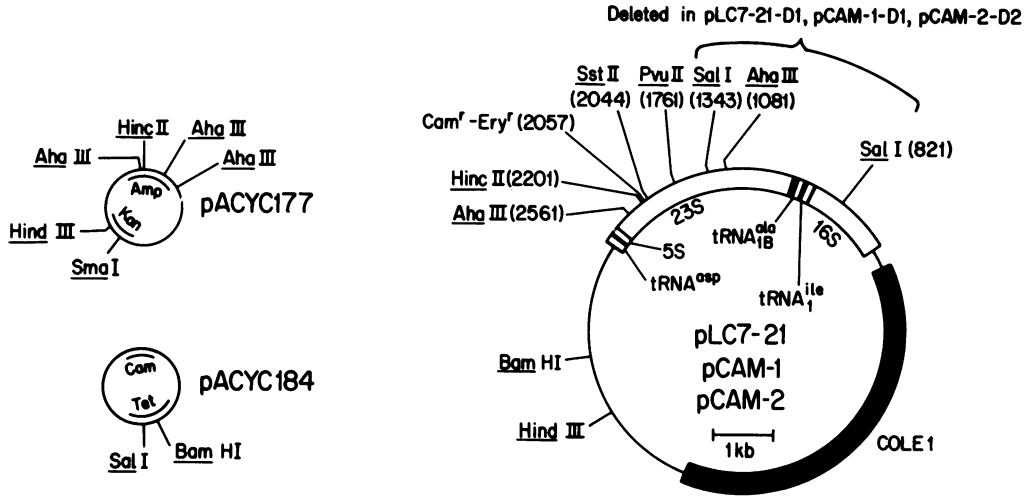

FIG. 1. Plasmid structures. pLC7-21, pCAM-1, and pCAM-2 are identical except for point mutations at the indicated positions. The only restriction nuclease recognition sites shown are the 6-base-pair specificity sites important for mapping the chloramphenicol-erythromycin resistance mutations. Numerical coordinates within the 16S and 23S RNA genes are described by a numbering system that begins at the first nucleotide of the mature RNA products of each gene.

plasmids pACYC177 and pACYC184. EM2 is F$^+$ *ilv-1 his-29 pro-2 tsx trpA9605 trpR ara*. Em348 (λ^- F$^-$ RecA$^+$ Tn*10*::*srl*) and EM349 (λ^- F$^-$ *recA56* Tn*10*::*srl*) are derivatives of W3110. EM363 (RecA$^+$ Tn*10*::*srl hsdR met thi-1* λ^- F$^-$) and EM364 (*recA56* Tn*10*::*srl hsdR met thi-1* λ^- F$^-$) are antibiotic-hypersensitive strains made by P1 transduction of Tn*10*::*srl* from MC1024 [*araD139* Δ(*ara-leu*)*7697* Δ(*lacZ*)M15 *galU galK rspL recA56* Tn*10*::*srl*] to the antibiotic-hypersensitive strain DB11 (24).

Isolation of mutations. Mutations in *rrnH* on pLC7-21 were isolated by using modifications of a previously described procedure (25). Briefly, EM2(pLC7-21) was mutagenized by placing a filter paper disk containing 70 μl of ethyl methane sulfonate on LB agar containing colicin E1 and 2.5 μg of chloramphenicol per ml after freshly spreading bacteria on the agar. This concentration of chloramphenicol is strongly but incompletely inhibitory to the growth of EM2(pLC7-21). Chloramphenicol-resistant mutants therefore formed prominent colonies on a weak lawn of strongly inhibited cells. After 2 days at 37°C, the cells were washed off the plate and mated overnight with strain W3110 Nal^r. The mating mixtures were then diluted with 20 volumes of LB agar containing colicin E1 and 20 μg of nalidixic acid per ml, followed by growth overnight at 37°C to allow W3110 Nal^r recipients to phenotypically express newly acquired chloramphenicol resistance mutations. Chloramphenicol-resistant pLC7-21 recipients were then selected on LB agar containing colicin E1, 20 μg of nalidixic acid per ml, and 3.5 μg of chloramphenicol per ml. The resulting colonies were purified by restreaking on the same medium and screened for colicin E1 resistance, sensitivity to phage BF23, and nutritional markers distinguishing between EM2 and W3110 Nal^r. Colonies testing as true chloramphenicol-resistant, colicin-immune W3110 Nal^r recipients were used as sources of purified plasmid DNAs. The plasmid DNAs were then tested for chloramphenicol resistance mutations as described below.

Mapping procedures. The genetic mapping procedures described below are similar to those previously described (24).

Recombinant DNA and DNA sequencing. Recombinant DNA procedures were performed according to Davis et al. (8). DNA was sequenced by using the dideoxynucleotide chain termination method and the single-stranded DNA phages M13mp8 and M13mp9 (34). DNA synthesis was primed by a 15-nucleotide primer obtained from P-L Biochemicals.

Antibiotic sensitivity testing. The sensitivities of bacterial strains to all antibiotics except streptogramin type B antibiotics were tested by streaking each strain on LB agar containing colicin E1 and the antibiotic to be tested. Closely spaced concentrations of antibiotics were used to insure testing of partially inhibitory and fully inhibitory antibiotic concentrations. Antibiotic sensitivity testing was performed by using the strains EM2, EM348, and EM349 and the antibiotic-hypersensitive strains EM363 and EM364. pCAM-1 and pCAM-2 caused increased chloramphenicol, oleandomycin, and erythromycin resistance in all strains.

Streptogramin type B antibiotics detectably inhibit only antibiotic-hypersensitive *E. coli* strains such as EM363 and EM364. Because only small amounts of streptogramin type B antibiotics are readily available, sensitivity was tested by placing a 6-mm-diameter filter paper disk containing 1 mg of the streptogramin type B antibiotic osteogrycin B on lawns of EM363 and EM364 freshly spread in a soft agar overlay on LB agar. Osteogrycin B causes a 2-mm zone of inhibition when these strains contain pLC7-21, pCAM-1, or pCAM-2.

RESULTS

Isolation of mutations. As described above, two chloramphenicol resistance mutations were independently isolated in bacterial strains containing *rrnH* on pLC7-21. The pLC7-21 derivatives in these two mutants are designated pCAM-1 and pCAM-2. To determine whether pCAM-1 and pCAM-2 contained mutations which confer chloramphenicol resistance, pCAM-1 and pCAM-2 DNAs were purified by density gradient centrifugation and used to transform the RecA$^+$ strains EM2, EM348, and EM363 and the RecA$^-$ strains EM349 and EM364. Transformants were selected on LB agar containing colicin E1 and then screened for chloramphenicol resistance by using many closely spaced concentrations of chloramphenicol to allow testing of partially inhibitory and completely inhibitory concentrations of the antibiotic. For all strains tested, pCAM-1 and pCAM-2 allowed growth on concentrations of chloramphenicol and

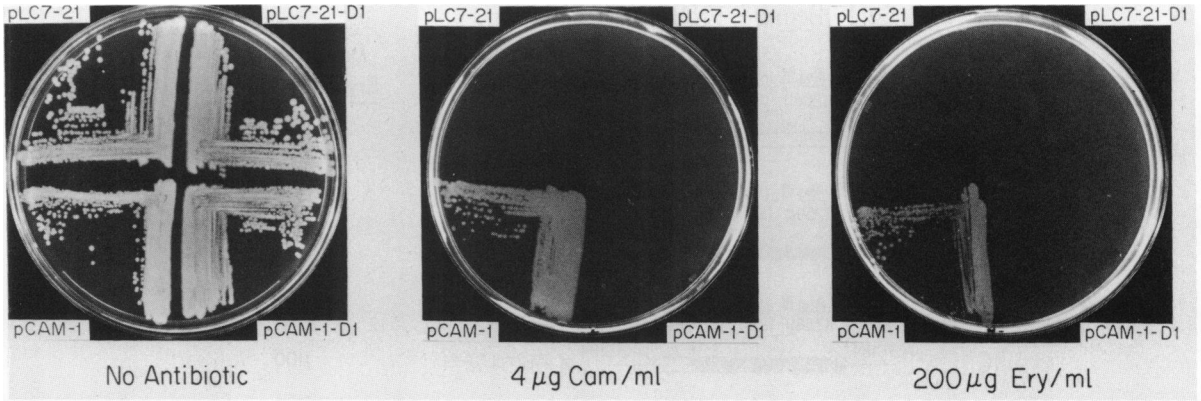

FIG. 2. Growth in the presence of antibiotics. Strains of EM2 which contain the indicated plasmids were streaked on LB agar containing colicin E1 and 4 μg of chloramphenicol per ml, 200 μg of erythromycin per ml, or no antibiotic. The cultures with no antibiotic were incubated for 24 h. The cultures with antibiotics were incubated for 48 h.

erythromycin which completely inhibited the growth of strains containing pLC7-21. The resistance phenotype of EM2(pCAM-1) on 4 μg of chloramphenicol per ml and 200 μg of erythromycin per ml [concentrations just sufficient to completely inhibit growth of EM2(pLC7-21)] are illustrated in Fig. 2. The resistance phenotype of EM2(pCAM-2) is similar to that of EM2(pCAM-1). In the course of these experiments, it became apparent that cells containing pLC7-21 are slightly more sensitive to chloramphenicol and erythromycin than cells containing no plasmid. Therefore, colicin E1 was added to the agar in all subsequent experiments to reduce survival of cells which have become slightly less antibiotic sensitive due to plasmid loss.

Resistance spectrum. The spectrum of antibiotic resistance conferred by pCAM-1 and pCAM-2 was tested using closely spaced antibiotic concentrations as described above. A number of $RecA^+$ and $RecA^-$ bacterial strains (see above) were used in these tests. The antibiotics tested included chloramphenicol, the macrolide antibiotics erythromycin, oleandomycin, niddamycin, tylosin, and spiramycin, the lincosamide antibiotics lincomycin and clindamycin, the streptogramin type B antibiotic osteogrycin B, the streptogramin type A antibiotic P114A, and two mixtures of synergistic streptogramin type A and B antibiotics (virginiamycin A plus B and mikamycin A plus B). When compared with cells containing pLC7-21, cells containing pCAM-1 and pCAM-2 were more resistant only to chloramphenicol and the 14-atom lactone ring macrolide antibiotics erythromycin and oleandomycin (Fig. 2; some data not shown). Cells containing pCAM-1 exhibited a degree of antibiotic resistance that is similar to that of cells containing pCAM-2. The fact that resistance to 14-atom lactone ring macrolide antibiotics is an unselected trait acquired by selection for chloramphenicol resistance strongly suggests that macrolide resistance is a frequent consequence of single mutational events in *rrnH* that cause chloramphenicol resistance. Since pCAM-1 and pCAM-2 confer resistance to chloramphenicol and 14-atom lactone ring macrolide antibiotics in $RecA^-$ strains, the chloramphenicol and macrolide resistance mutations in *rrnH* on these plasmids must be dominant or codominant to the seven chromosomal *rrn* operons which code for sensitive ribosomes.

Internal *rrnH* deletions. To verify that the mutations in pCAM-1 and pCAM-2 are in the *rrnH* operon, *Sal*I restriction nuclease fragments internal to *rrnH* were removed from pCAM-1, pCAM-2, and pLC7-21 (Fig. 1). The resulting plasmids (pCAM-1-D1, pCAM-2-D1, and pLC7-21-D1) do not confer chloramphenicol or erythromycin resistance (Fig. 2; some data not shown). Therefore, the chloramphenicol-erythromycin resistance mutations in pCAM-1 and pCAM-2 are located in *rrnH*. These experiments (data not shown) also showed that the removal of *Sal*I fragments from *rrnH* on pLC7-21 does not alter the antibiotic sensitivity conferred by this plasmid (see above). Therefore, the increased sensitivity resulting from pLC7-21 is probably not due to the expression of the 16S or 23S RNA genes of the wild-type *rrnH* operon on pLC7-21.

Mapping the mutations. Previously described methods for fine-scale genetic mapping of dominant or codominant mutations in *rrn* operons (24) were used to map the mutations in pCAM-1 and pCAM-2. The mapping methods used are based on plasmid-plasmid marker rescue experiments. In plasmid-plasmid marker rescue experiments, restriction fragments to be tested for the Ery^r and Cam^r mutations of pCAM-1 or pCAM-2 were cloned into pACYC177 or pACYC184 (Fig. 1). The cloned fragments to be tested did not by themselves confer erythromycin or chloramphenicol resistance because they contain only partial *rrnH* structural genes or lack the *rrnH* promoter region. The resulting pACYC derivatives were transformed into EM2(pLC7-21). Overnight broth cultures of individual transformants were then plated on LB agar containing colicin E1 and 200 μg of erythromycin per ml. If the fragment of pCAM-1 or pCAM-2 (cloned into the pACYC plasmid) contained the Ery^r mutation, the mutation could be transferred by recombination to pLC7-21, resulting in synthesis of Ery^r ribosomes and the emergence of Ery^r cells. A restriction fragment cloned into the pACYC plasmid was therefore judged to have the mutation responsible for erythromycin resistance when more colonies grew on agar containing erythromycin than grew from control cultures containing the same fragment of pLC7-21 cloned in the pACYC plasmid. Several independent transformants were always examined by this method to insure that differences in the number of Ery^r colonies were not merely the result of statistical fluctuations.

The *Sal*I-*Bam*HI fragment containing the distal end of the 23S RNA gene (Fig. 1 and 3) was determined by plasmid-plasmid marker rescue experiments to have the Ery^r mutation of pCAM-1 and pCAM-2 (Fig. 3). Hybrid *Sal*I-*Bam*HI fragments were then constructed by using the *Sst*II sites at position 2044 of the 23S RNA genes (Fig. 1 and 3). The resulting hybrid plasmids have pLC7-21 DNA on one side of the *Sst*II sites and either pCAM-1 or pCAM-2 DNA on the other side (Fig. 3). The hybrid plasmids were then tested for

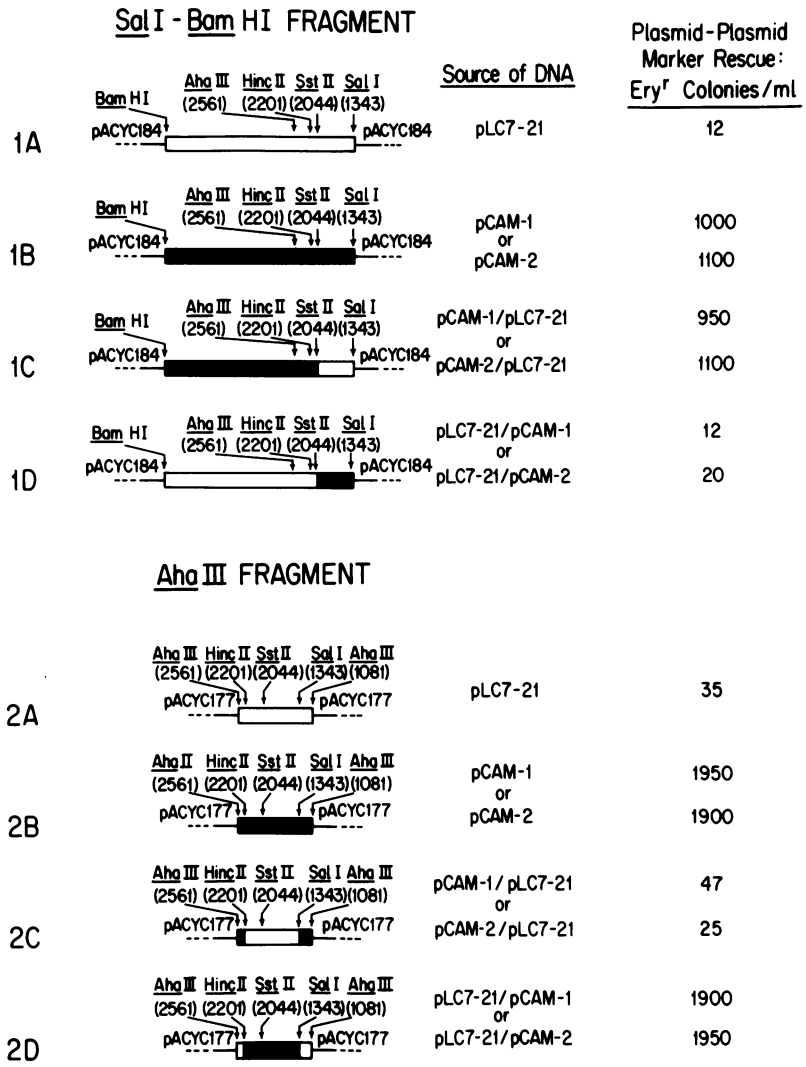

FIG. 3. Diagrams illustrating the structures of plasmids important to the final stages of mapping the Cam^r-Ery^r mutations in pCAM-1 and pCAM-2. The results of these mapping experiments prove that the Ery^r and Cam^r mutations of pCAM-1 and pCAM-2 lie within a 154-base-pair region between the *Sst*II (position 2044) and *Hin*cII (position 2201) recognition sites in the 23S rRNA genes.

the presence of the Ery^r mutation by plasmid-plasmid marker rescue experiments. The results (Fig. 3) prove the Ery^r mutations of both pCAM-1 and pCAM-2 are located downstream of the *Sst*II sites at position 2044 of the 23S RNA genes.

To further map the mutations, shotgun recombination mapping methods (24) were used. Briefly, a mixture of *Aha*III fragments of pCAM-1 or pCAM-2 was ligated into *Aha*III-digested pACYC177 (Fig. 1), and the resulting mixture of hybrid plasmids was transformed into EM2(pLC7-21). Transformants were selected on LB agar containing colicin E1 and 20 μg of kanamycin per ml. After incubation for 48 h to allow recombination and phenotypic expression of mutations, the transformants were replica plated onto LB agar containing colicin E1, 20 μg of kanamycin per ml, and 200 μg of erythromycin per ml. A fraction of the transformant colonies grew after replica plating onto medium containing erythromycin because the pACYC derivatives in those colonies contained the *Aha*III fragments which had the Ery^r mutations of pCAM-1 or pCAM-2, and these pACYC177 derivatives had participated in plasmid-plasmid marker rescue with pLC7-21 during colony growth before replica plating onto medium containing erythromycin. pACYC177 derivatives containing the *Aha*III fragment with the Ery^r mutations of pCAM-1 and pCAM-2 were therefore selectively recovered in the colonies which grew after replica plating. Mixtures of pLC7-21 and pACYC177 derivative plasmid DNAs were then purified from these colonies, and new transformants containing only the pACYC177 derivative were isolated. The identity of the cloned *Aha*III fragment was then determined by restriction nuclease digestion of purified pACYC177 derivative DNA. pACYC177 derivatives with *Aha*III fragments containing DNA between positions 1081 and 2561 of the 23S genes of both pCAM-1 and pCAM-2 were isolated by this procedure and therefore contain the Ery^r mutations. A useful feature of the shotgun recombination mapping procedure is the fact that the mutation is usually recovered on the fragment cloned into the pACYC plasmid (24). Therefore, the *Aha*III fragment from position 1081 to 2561 of the 23S RNA gene of pLC7-21 was also cloned into pACYC177, and plasmid-plasmid marker rescue experiments were used to confirm the presence of the Ery^r mutations in the cloned *Aha*III fragments of pCAM-1 and pCAM-2 (Fig. 3).

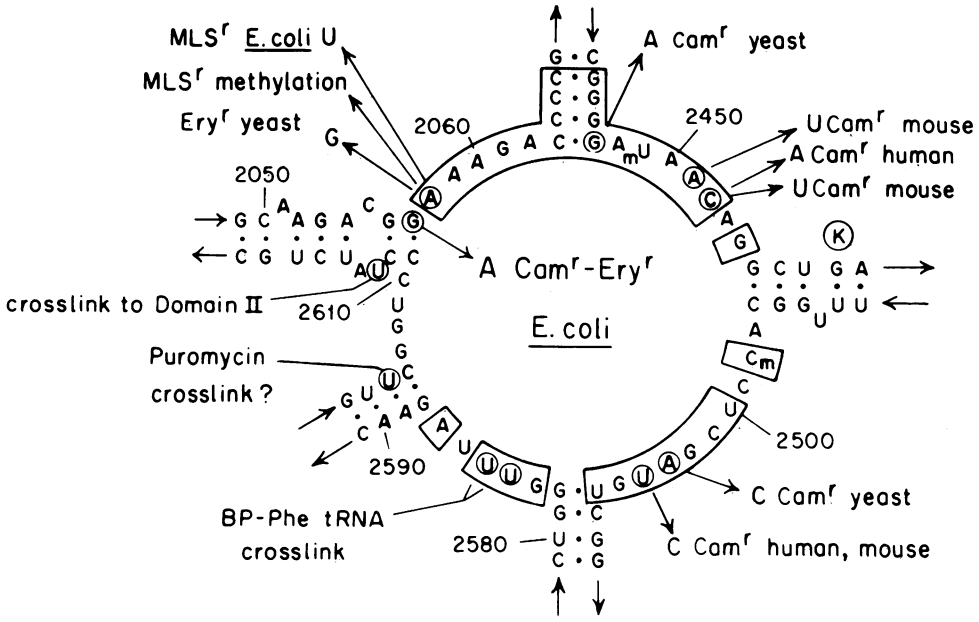

FIG. 4. Secondary structure of a portion of *E. coli* 23S RNA (22). Previously characterized mutations or interactions (1, 2, 9, 10, 16, 24–29) affecting *E. coli* 23S RNA or homologous regions of rRNA from other organisms are circled and identified on the periphery of the secondary structure. The mutation characterized in this paper is circled and identified on the inside of the secondary structure. A circled K indicates a guanosine protected from kethoxal modification by subunit association (14). The boxed-in regions of sequence are conserved during evolution (22).

Hybrid *Aha*III fragments were then constructed by using a *Hin*cII sites at positions 1343 and 2201 in the 23S RNA genes (Fig. 3). The resulting hybrid fragments were then tested for the presence of the Ery^r mutation by plasmid-plasmid marker rescue experiments. The results (Fig. 3) prove that the Ery^r mutation is located between the *Hin*cII sites at positions 1343 and 2201 of the 23S RNA genes of both pCAM-1 and pCAM-2.

The mapping experiments described above and in Fig. 3 prove the Ery^r mutations of pCAM-1 and pCAM-2 are located between the *Sst*II sites at position 2044 of the 23S RNA genes and the *Hin*cII sites at position 2201 of the 23S RNA genes. To determine whether the Cam^r mutations of pCAM-1 and pCAM-2 are also in this region, at least 25 recombinants resulting from each plasmid-plasmid marker rescue experiment described in Fig. 3 were restreaked on LB agar containing colicin E1 and 200 μg of erythromycin per ml. The purified Ery^r colonies were then tested for chloramphenicol resistance (Fig. 2). All Ery^r recombinants from successful plasmid-plasmid marker rescue experiments tested as Cam^r, proving the Cam^r mutations of pCAM-1 and pCAM-2 are also located between the *Sst*II sites at position 2044 and the *Hin*cII sites at position 2201. The few Ery^r colonies arising in plasmid-plasmid marker rescue experiments with cloned fragments from pLC7-21 (Fig. 3) did not test as Cam^r, consistent with their origin by de novo mutation.

Sequencing the mutations. DNA fragments of pCAM-1, pCAM-2, and pLC7-21 between *Tha*I sites at position 2045 of the 23S RNA genes and *Hin*cII sites at position 2201 of the 23S RNA genes were cloned into the single-stranded DNA phages M13mp8 and M13mp9 and sequenced in both directions by using the dideoxynucleotide chain termination method (sequencing gels not shown). The *rrnH* sequence from pLC7-21 was identical to the sequence of *rrnB* (3) in this region. Both pCAM-1 and pCAM-2 have a guanine-to-adenine transition at position 2057 of their 23S RNA genes. Since the Ery^r and Cam^r mutations of pCAM-1 and pCAM-2 map within the regions sequenced, a guanine-to-adenine transition at position 2057 of the 23S RNA gene of *rrnH* causes resistance to chloramphenicol and 14-atom lactone ring macrolide antibiotics.

DISCUSSION

Resistance to chloramphenicol and 14-atom lactone ring macrolide antibiotics can result from a guanine-to-adenine transition at position 2057 of the *rrnH* 23S RNA gene. This mutation causes a sequence alteration in a region of 23S RNA secondary structure that is also affected by mutations and posttranscriptional modifications causing resistance to chloramphenicol and the macrolide, lincosamide, and streptogramin type B antibiotics (Fig. 4). The mutational alteration described in this paper provides genetic support for the already strong conclusion (see the legend to Fig. 4) that the secondary structure depicted in Fig. 4 exists in 23S RNA in the ribosome.

The mutation described in this paper and the previously isolated mutations described in Fig. 4 demonstrate that antibiotics acting on the peptidyl transferase region have both similarities and differences in binding sites or modes of action. The differences and overlaps of the resistance phenotypes caused by the alterations of 23S RNA are reflected by the differences and similarities of the inhibitory properties of the antibiotics (reviewed above) and by the competition of many of these antibiotics for binding to the 50S ribosomal subunit (6, 11, 32).

There is strong evidence that the region of 23S RNA depicted in Fig. 4 is part of in the peptidyl transferase region. As reviewed above, mutations in this region cause resistance to antibiotics which inhibit the peptidyl transferase region of the 50S ribosomal subunit. Very strong evidence that the peptidyl transferase active site is near this region is also

provided by the observation that the compound 3-(4'-benzoylphenyl)propionyl phenylalanyl-tRNA (BP-Phe-tRNA) can be bound in the P site of intact ribosomes, photo-cross-linked to the region of 23S RNA shown in Fig. 4, and still retain its ability to participate in peptide bond formation (1). Puromycin, an aminoacyl-tRNA analog that binds to the A site of the ribosome and participates in peptide bond formation catalyzed by the peptidyl transferase active site, can be photo-cross-linked to a region of 23S RNA that contains the sequence . . .GUUCG. . .. One of the three . . .GUUCG. . . sequences in 23S RNA is also in the region of secondary structure depicted in Fig. 4. Therefore, a large body of evidence strongly indicates that 23S RNA is an important component of the peptidyl transferase region. It therefore seems worthwhile to entertain the notion that 23S RNA is the peptidyl transferase enzyme. As a test of this hypothesis, we suggest that the use of BP-Phe-tRNA photo-cross-linked to 23S RNA (see above) would assist demonstration of peptidyl transferase activity by purified 23S RNA-BP-Phe-tRNA by facilitating alignment of the putative peptidyl transferase active site and one of the two required substrates. Another advantage of this approach is the fact that a sensitive and specific assay for peptide bond formation would be available because the other required substrate would become covalently linked to 23S RNA through BP-Phe if peptide bond formation occurs. The successful demonstration of peptide bond formation catalyzed by purified 23S RNA would have important implications regarding the order of appearance of proteins and nucleic acids during prebiotic evolution and would illuminate the observation that ribosomes have RNA components.

The results presented previously (20, 24, 25) and in this paper demonstrate that resistance to spectinomycin, chloramphenicol, macrolide, lincosamide, and streptogramin type B antibiotics can be observed when only a portion of the ribosomes contain RNA of the resistant type. Our unpublished experiments (C. D. Sigmund and E. A. Morgan) demonstrate that the degree of resistance increases when the proportion of resistant-type *rrn* operons increases, and that mutant *rrn* operons present in a single copy per cell on a lysogenic lambda specialized transducing phage confer detectable, albeit weak, erythromycin and spectinomycin resistance phenotypes. Therefore, resistance to these bacteriostatic antibiotics appears to be codominant with sensitivity. We have previously suggested (20) that the conclusion that antibiotic resistance mutations in r-protein genes are recessive (18, 30, 35, 36) may be true for some antibiotics only because resistant-type r-proteins cause defects in ribosome assembly or function. We therefore propose that it is correct to consider spectinomycin, chloramphenicol, macrolide, lincosamide, and streptogramin type B resistance mutations in ribosomal genes as codominant with sensitivity when sensitive- and resistant-type ribosome components both make substantial contributions to correct and appropriate protein synthesis, although strict codominance is unlikely because ribosome synthesis is autocatalytic and ribosomes synthesize all other synthetic machinery of the cell. The phenomenon of mRNA blockage previously invoked to explain the apparent dominance of sensitivity in sensitive and resistant r-protein gene merodiploids (18, 30, 32) may also affect the observed codominance. Despite the fact that strict codominance is unlikely, we believe the term codominance is justified for mutations that confer resistance to spectinomycin, chloramphenicol, macrolide, lincosamide, and streptogramin type B antibiotics because it is clear from our results that antibiotic-inhibited sensitive ribosomes in sensitive and resistant rRNA gene merodiploids do not strongly poison protein synthesis by resistant ribosomes and do not poison any other cellular process.

The mutations in rRNA genes described previously (20, 24, 25) and in this paper demonstrate that rRNA plays a role in the action of a large percentage of medically useful ribosome-active antibiotics. Medically important antibiotic resistance might therefore result from antibiotic target site alterations of rRNA. Naturally occurring, clinically and commercially important, MLS resistance resulting from methylation of 23S RNA at position 2058 (see Fig. 4) in fact presaged the isolation of an MLS resistance mutation at position 2058 of 23S RNA (see Fig. 4). It is therefore possible that the spectinomycin resistance mutation in a 16S RNA gene (20, 24) and the chloramphenicol-erythromycin resistance mutation in a 23S RNA gene (described in this paper) foreshadow the discovery of naturally occurring spectinomycin or chloramphenicol-erythromycin resistance resulting from post-transcriptional modifications of rRNA. We speculate that target site modifications of this type may be the ultimate resistance mechanism adapted by microorganisms after repeated challenge with antibiotic derivatives that defeat other resistance mechanisms.

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