ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1995, p. 797–805 Vol. 39, No. 4
0066-4804/95/$04.00+0

MINIREVIEW

Insights into Erythromycin Action from Studies of Its Activity as Inducer of Resistance

BERNARD WEISBLUM*

Department of Pharmacology, University of Wisconsin Medical School, Madison, Wisconsin 53706

INTRODUCTION

Appreciation of the inducibility of erythromycin resistance began as an observation in the clinical bacteriology laboratory during susceptibility testing of erythromycin-resistant clinical isolates of *Staphylococcus aureus*. It was noted that inhibition zones surrounding spiramycin, lincomycin, and pristinamycin I (streptogramin B family) test disks placed close to an erythromycin test disk deviated from the expected circular shape and assumed a distorted "D" shape instead. Such observations suggested a possible antagonistic interaction between erythromycin, on the one hand, and spiramycin, lincomycin, or pristinamycin I, on the other (5, 7). The interaction turned out to be a functional rather than a physical antagonism, and out of these observations grew the notion of erythromycin-inducible resistance toward erythromycin, initially (41, 55), and then, more generally, toward all the macrolide, lincosamide, and streptogramin type B (MLS) antibiotics (57).

Vazquez (50) and Vazquez and Monro (52) showed that antibiotics belonging to each subclass of the MLS antibiotics competed with chloramphenicol for uptake by intact cells. Competition for binding to purified 50S ribosome subunits was shown only for macrolides and lincosamides but not for streptogramin type B antibiotics. Collectively, these observations suggested that an alteration of 50S subunit function was involved in resistant cells. In a study of the time and concentration dependence of induction, Weisblum et al. (58) showed that (i) the optimal erythromycin concentration for induction was between 10 and 100 ng/ml, the threshold of its inhibitory action, (ii) at the optimal inducing concentration of erythromycin cells became phenotypically resistant within 40 min, and (iii) ribosomes from induced cells apparently bound labeled erythromycin and lincomycin with a reduced affinity. By mixing ribosome preparations from susceptible and resistant cells and noting no loss of expected antibiotic binding activity, it was possible to exclude the alteration of the antibiotic by modifying enzymes present as contaminants in the ribosome preparation.

Consistent with this picture, Allen (1) showed that cell extracts of resistant *S. aureus* carried out erythromycin-resistant protein synthesis in vitro, suggesting that a component of the protein-synthesizing machinery had been altered. A posttranscriptional methylation of a single adenine residue in 23S rRNA, comprising the induced biochemical alteration (30, 31), was located at *Escherichia coli* coordinate 2058 (A-2058) (47), and translational attenuation (16, 24), the mechanism for its regulation, was proposed. As discussed below, this unusual mechanism of gene regulation requires no repressor proteins but, instead, is based on the conformational isomerization of the *ermC* message to a translationally active form. How might this be achieved?

ERYTHROMYCIN AS A REGULATOR OF *ermC* EXPRESSION

A clue to the mechanism of *erm* regulation came from a comparison between the inhibitory and inducing potencies of a set of erythromycin analogs and derivatives. Pestka et al. (42) concluded that the action of erythromycin as an inducer of *erm* expression was inseparable from its inhibitory action on ribosomes: "Only derivatives with antibacterial activity induced resistance, although some antibacterial compounds did not induce resistance. No derivatives without inducing activity but with ability to induce resistance were found." Allen (2) also noted that several erythromycin derivatives, although active as inhibitors, were inactive as inducers, from which he concluded that "distinct binder/receptor sites are utilized for inhibition of ribosome function and induction of resistance." Although Allen came to a different conclusion, his observations actually supported the simpler unifying conclusion of Pestka et al. (42) that linked inducing and inhibitory activities.

The mechanistic significance of the observation of Pestka et al. (42) was not fully appreciated when it was first published; however, it is now clear how it provides the key to understanding the mechanism of induction. Erythromycin makes its presence felt as an inducer by interacting with susceptible ribosomes (18, 25). The erythromycin-ribosome complex, in turn, makes its presence felt during induction by inhibiting translation of a 19-amino-acid peptide encoded by the 141-nucleotide leader sequence of the *ermC* message extending from the transcription initiation site to the methylase initiator Met codon.

Conformational isomerization of the *ermC* leader sequence. The 141-nucleotide *ermC* mRNA leader can assume at least three alternative conformations, as shown in Fig. 1. In its nascent form, the leader would be expected to assume the conformation shown in Fig. 1A. The pattern of association shown was assigned on the basis of the temporal order of synthesis of complementary segments. Thus, in the uninduced "ground state" shown in Fig. 1A, segment 2 associates with segment 1, and segment 4 associates with segment 3. With the *ermC* message in this conformation, translation of the ErmC protein is initiated with a low efficiency because the first two codons of ErmC, AUG and AAU (fMet Asn), as well as the ErmC ribosome binding site, are sequestered by secondary structure. Induction provides conditions that favor a translationally active conformation of the *ermC* message shown in

* Mailing address: Department of Pharmacology, University of Wisconsin Medical School, 1300 University Ave., Madison, WI 53706. Phone: (608) 262-0972. Fax: (608) 262-1257. Electronic mail address: weisblum@macc.wisc.edu.

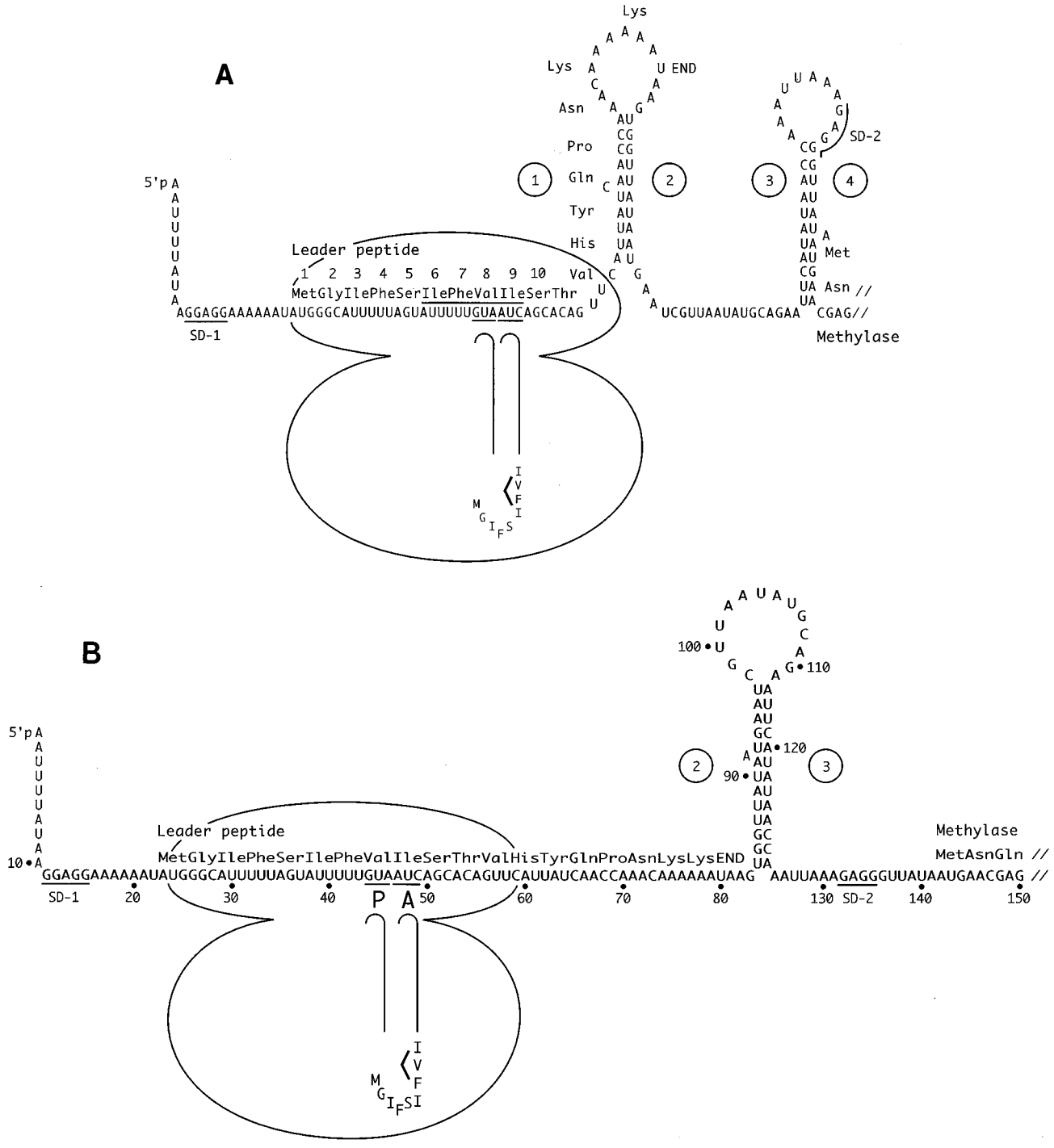

FIG. 1. Proposed conformational transitions of the *ermC* leader sequence during induction. (A) Conformation of *ermC* mRNA leader sequence early during induction by erythromycin. The four major segments of the *ermC* attenuator are paired as segments 1:2 and segments 3:4, reflecting the temporal order of their synthesis. The ribosome is shown stalled during the addition of Ile-9 to the growing leader peptide. See text for details. The extent to which the ribosome covers the leader sequence upstream of this site is supported by in vivo footprinting experiments (34). On the downstream side, the stalled ribosome is shown disrupting segments 1:2 at CAU, the codon for His-12. This is based on the experimental finding (25) that a mutation C59A (His-12-Asn) leading to a mismatch at that position resulted in constitutive expression of *ermC*. (B) Conformation of the *ermC* mRNA leader sequence in its fully induced state. As a consequence of stable complex formation, the erythromycin-ribosome complex, and the *ermC* message, the association between segment 1 and segment 2 is prevented. This favors the association between segments 2 and 3, which uncovers the ribosome binding site and first two codons of the ErmC methylase encoded by segment 4. (C) Inactive conformation of the *ermC* mRNA leader sequence resulting from either removal of erythromycin or another inducer or from maximal methylation of 23S rRNA and a maximum concentration of resistant ribosomes. The transition from the conformation shown in Fig. 2B to that shown in Fig. 2C requires only that segment 4 associate with segment 1. A return to the ground state shown in Fig. 2A would require activation energy to dissociate segments 2:3, which would then enable a return to segments 1:2 and segments 2:3.

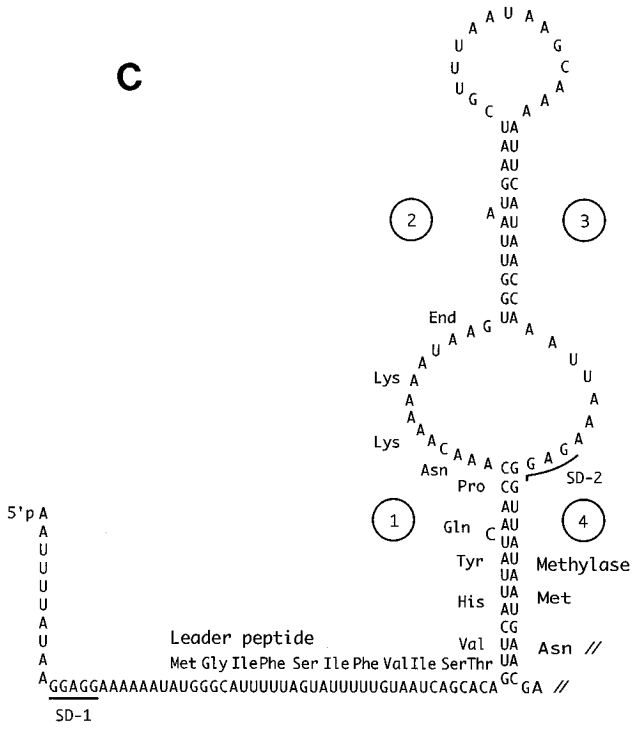

FIG. 1—*Continued.*

Fig. 1B. Induction of *ermC* starts with the binding of erythromycin to ribosomes that synthesize a 19-amino-acid peptide, MGIFSIFVISTVHYQPNKK, encoded by the *ermC* mRNA leader upstream of the ErmC open reading frame, and culminates with an increased efficiency of ErmC translation owing to the conformational isomerization of its message to the active form shown in Fig. 1B.

Thus, for erythromycin to act as an inducer it must inhibit synthesis of a peptide whose composition and location as an open reading frame are critically defined and located in relation to the conformation of the *ermC* leader sequence. Because the ribosome must pause while it is translating the leader peptide, the process of induction based on this principle is generally called "attenuation," and because the regulatory signal directly affects translation, the mechanism of regulation is therefore referenced as "translational attenuation." This mechanism contrasts with transcriptional attenuation, in which the ribosomal pause is linked functionally to antitermination, another process in which the conformation of the message is linked to its expression. How transcriptional attenuation serves to regulate amino acid biosynthesis has been reviewed (28). We have studied one system, *ermK* regulation, in which it appears that expression may be regulated by transcriptional attenuation (29).

A functionally useful mechanism of gene regulation should contain negative feedback features that would down-regulate gene expression when it is no longer needed. This down-regulation can be in response either to a reduction in the original inductive stimulus, i.e., erythromycin concentration, or to saturation of the cell with the products of induction, i.e., methylated ribosomes. The optimal function of the latter mechanism should result in down-regulation of gene expression even during a continued presence of the inducer. Thus, after the inducing concentration of erythromycin has been reduced, e.g., by dilution, and can no longer support induction, the leader region can refold to assume an inactive conformation, shown in Fig. 1C. The conformational transition 1B to 1A would also repress *ermC*, but energy would first be required to dissociate stem 2:3. In contrast, the conformational transition 1B to 1C would not require additional energy; it would be expected to be exothermic, and would therefore be favored.

The translational attenuation model predicts that the synthesis of methylase should eventually become self-limiting since a critical concentration of susceptible ribosomes is needed to sustain induction. Results that support this aspect of the model were obtained by Gryczan et al. (18), who reported that they were unable to induce *ermC* in an oleandomycin-resistant mutant of *Bacillus subtilis*. The mutant carried a chromosomal mutation, *ole-2*, presumed to affect a ribosomal protein constituent of the 50S subunit. Moreover, inactivation of the methylase structural gene by in vitro deletion of several codons yielded in *B. subtilis* an inactive methylase that appeared to be synthesized at an abnormally high rate following induction by erythromycin (46). This interesting phenomenon was ascribed to an intracellular maximal level of susceptible ribosomes resulting, in turn, in maximal expression of the induced phenotype. In later studies, Denoya et al. (10) and Breidt and Dubnau (6) proposed that ErmC additionally acts as a translational repressor, that its probable binding site on the *ermC* message overlaps the ErmC ribosome binding site, -GGAG-, and that this specificity was related to the rRNA sequence that contains A-2058, GAAAG. Kinetic arguments, including studies of a mutant, *ermC′*, that lacks enzymatic activity but that apparently can function as a repressor, were proposed in support of the model. The footprint that the bound ErmC would leave on its message, however, has not yet been demonstrated experimentally.

***ermC* leader peptide.** The 19-amino-acid leader peptide-coding sequence plus its stop codon, 60 nucleotides in length, thus provides a region for the ribosome to pause while under the influence of erythromycin. What aspects of the leader peptide structure allow it to function optimally in induction? Will any 19-amino-acid peptide work? If not, which amino acids are critical for this function, and where within the leader peptide are they located? How is the length of the leader peptide related to its optimal function? These questions will be discussed by considering the effects of mutations sequentially, beginning from the 5′ end of the message.

The use of homopolymeric mRNA-coding sequences synthesized with polynucleotide phosphorylase led to the observation that ribosomal synthesis of polylysine and polyproline directed by polyadenylic acid and polycytidylic acid, respectively, was highly susceptible to erythromycin, whereas synthesis of polyphenylalanine directed by polyuridylic acid was not (50). The results of these studies supported the erroneous generalization that incorporation of hydrophilic amino acids into protein was more susceptible to the inhibitory action of erythromycin than was the incorporation of hydrophobic amino acids. This generalization led Gryczan et al. (16) to assign to the lysine residues at positions 18 and 19 (Lys-18 and Lys-19, respectively) the key role in determining where the ribosome pauses during induction. As shown below, the part of the leader peptide that determines susceptibility to inhibition by erythromycin is not a single amino acid but an amino acid context consisting of four or five amino acid residues.

Because of its small size, the 19-amino-acid *ermC* leader peptide provides a useful model protein in which to study the detailed action of erythromycin as an inhibitor of protein synthesis. (i) The leader peptide is long enough that it can provide a variety of prospective amino acid sequences within which inhibition of protein synthesis can be studied. (ii) It is also

short enough that a systematic modification of its sequence can be undertaken in order to locate precisely the factors that influence the efficiency of induction, and therefore might be related to the detailed action of erythromycin as well. (iii) The linkage of protein synthesis inhibition to gene expression provides the components needed to construct a translational fusion with an easily scored reporter gene, *lacZ*. (iv) The functional linkage between inhibition and induction can provide additional details of the action of erythromycin at the molecular level, e.g., how closely must the ribosome approach a stem-loop structure to perturb its integrity. (v) The structural linkage of the *ermC* attenuator to a reporter gene, i.e., by translational fusion to *lacZ*, provides two stages of amplification events (induced lacZ synthesis and lacZ activity amplification events, i.e., induced LacZ synthesis and LacZ catalytic activity).

***ermC* leader mutations that result in constitutive expression.** Mutational alterations in the leader region of the *ermC* message have provided a wealth of confirmatory data for the translational attenuation model. Thus, a spontaneous point mutation in stem segment 1 would be expected to destabilize segments 1:2 allowing segments 2:3 and unassociated segment 4 to form; a mutation in either segment 3 or 4 would directly destabilize segments 3:4, leading to increased *ermC* expression. Finally, a spontaneous deletion of 59 nucleotides that encode the 19-amino-acid leader peptide would also cleanly delete segment 1, allowing formation of segments 2:3 and an unassociated segment 4, also leading to increased *ermC* expression, (25).

The model predicts, as has been observed experimentally, that mutations leading to constitutive expression would not be found to involve segment 2 because such mutations would destabilize both segments 1:2 and segments 2:3, leaving segments 3:4, associated, as in the local conformation found in the repressed state. On the other hand, mutations to constitutive expression should occur in the other three segments; nucleotide changes in segment 1 would free up segment 2 to pair efficiently with segment 3, thereby freeing segment 4, leading to the efficient initiation of methylase synthesis. Single nucleotide changes in segment 3 or 4 would directly disrupt the association of 3:4, likewise leading to the efficient initiation of methylase synthesis. Hahn et al. (22) constructed a set of deletions in which segments 1, 1-2, and 1-2-3 were progressively deleted. Inducibility in these mutants showed "on, off, on" expression, respectively, which would be expected.

The spontaneous constitutively resistant mutants described above were selected with either lincomycin, carbomycin, or tylosin; the rationale for the use of these antibiotics was that they have no inducing activity and that survival in their presence would be assured if the inducible methylase were constitutively expressed. A constitutive mutant of *ermM* (very similar to *ermC*) was isolated by Lampson and Parisi (32) from a clinical specimen in which a leader sequence containing only segment 4 of the *ermC* attenuator appeared to be present. The finding of such a mutant in a clinical sample suggests that pressure for constititive expression similar to that used in the laboratory operates in the clinic as well. The clinical implications of this observation will be discussed in further detail below.

Relation of *ermC* induction to the molecular action of erythromycin. Two important attributes of the leader peptide relevant to induction are its size and the amino acids of which it is composed. Although the leader peptide is 19 amino acids in length, only the first 9, MGIFSIFVI-, are necessary for induction because replacement of TCA–Ser-10 with TAA-Och remains inducible, whereas the same substitution at Ile-9 is not (36). That at least part of the leader peptide must be synthesized was reported by Dubnau (14), who replaced GGC–Gly-2 with UAA-Och in the leader peptide and found that cells carrying the resultant construction could not be induced. Additional alterations of leader peptide amino acids upstream of Ile-9, namely, Ile-6, Phe-7, and Val-8, showed that they were also critical for induction because most alterations in these amino acid residues resulted in the loss of inducibility (36). Moreover, ribosome footprinting experiments showed erythromycin-dependent protection of the *ermC* mRNA sequence that encodes these amino acids; no protection was seen in the sequence immediately downstream that encodes stem segment 1, whose accessibility to dimethylsulfate actually increased during induction, in accordance with the model.

From these studies it was learned that the ribosome, stalled at Ile-9 (corresponding to nucleotides 47 to 49 of the leader), can destabilize the secondary structure associations of nucleotides 56 to 97 (stem 1:2), despite the absence of any protection demonstrable by footprinting at this location. Thus, the ribosome does not leave a footprint directly on the entire sequence, whose internal association it prevents or disrupts. It is probably not necessary to disrupt the secondary structure of stem segment 1 beyond C-59, located in the codon for CAU–His-13, because a mutation, C-59→A, that would destabilize segments 1:2 at precisely this location (by apposing A to G) was found in a constitutively resistant mutant (25).

Gryczan et al. (16) proposed the codons for Lys-18 and Lys-19 of the leader peptide as the site at which the ribosome pauses during induction; this implies the unlikely possibility that ribosomes containing bound erythromycin can elongate a nascent peptide chain to the extent of 18 to 19 amino acid residues. The studies of Contreras and Vazquez (8) and of Vazquez (51a) suggested that erythromycin inhibits elongation of the nascent peptide when it reaches a length of about two to five residues; our studies showed that the critical inhibition must occur before Ile-9, probably between Ser-5 and Val-8 (34–36). These experimental data showed that a ribosome did not have to translate beyond Ser-10 because placement of a UAA stop codon at that position did not abolish induction, but additionally, a ribosome complexed with erythromycin would not even be able to translate as far as Lys-18 and Lys-19. Even if the ribosome did translate the leader peptide as far as Lys-18 or Lys-19 and stalled, it would be expected, as a result, to repress rather than induce *ermC* because by pausing at this location, the ribosome would occlude segment 2, favoring the stabilization of paired segments 3:4, and thereby the repressed state. The conclusions of Contreras and Vazquez (8) are also consistent with the in vivo data (34) that showed a footprint of the ribosome over codons 4 to 9 and no apparent protection of the nucleotides that encode Lys-18 and Lys-19, which were maximally accessible to DMS, irrespective of induction.

Narayanan and Dubnau (39) attempted to characterize the association of erythromycin-ribosome complexes with *ermC* mRNA by use of a mapping technique based on protection of the *ermC* leader against an endogenous nuclease(s) of unknown identity or specificity. From the pattern of protection that they observed, they inferred that the ribosome stall site was located upstream of nucleotide 80. Since nucleotide 80 occurs within the codon for Lys-19, the last leader peptide amino acid, their conclusion implies, at most, that the erythromycin-ribosome complex pauses somewhere within the 57-nucleotide *ermC* leader peptide-coding sequence.

Still lacking is an explicit model that explains how the amino acid context of the critical region of the *ermC* leader peptide contributes to induction. In this respect, induction may reflect a distinctly novel phenomenon whose rules have not yet been

defined. Assuming that one starts with a leader peptide mutant that is not inducible, e.g., Val-8–Leu, it has been shown that erythromycin fails to stabilize the *ermC* message and fails to leave its footprint in the presence of erythromycin (cf. Fig. 4 in reference 34). Can one find amino acid replacements (suppressors) at other sites in the leader peptide that will restore inducibility? If so, which factor is compensated: charge, shape, volume, or hydrophobicity?

The range of amino acid replacements in the leader peptide that influence induction requires that additional types of interactions involving ribosome, antibiotic, and nascent peptide be considered. One possibility would be that the nascent peptide interacts directly with the antibiotic-ribosome complex. Perhaps the context-complexity of the critical leader peptide region is combinatorial in nature and acts by placing two amino acid side chains, four residues apart, in apposition on the same face of an alpha-helix. Such an interpretation would follow from models proposed by Yonath et al. (59) and Ryabova et al. (43), according to which the first few amino acids in the nascent peptide are confined to a tunnel or channel in which they assume, at least temporarily, an alpha-helical conformation. Moreover, the apparent ineffectiveness of amino acid alterations at Gly-2 and Ile-3 (fMGIFSIFVISTV-) at influencing induction is consistent with the conclusions of Contreras and Vazquez (8) and of Vazquez (51a) that erythromycin only affects synthesis of the growing peptide chain after it has reached a critical length of two to five residues.

That the functional target of erythromycin action was something other than peptide bond formation per se was demonstrated by Cundliffe and McQuillen (9). They pretreated a cell-free protein-synthesizing system with chlortetracycline so that the aminoacyl site (A-site) was clear and that all nascent peptides were in the peptidyl site (P-site). Such a system can form peptide bonds with puromycin, and the synthesis of such peptide bonds was shown to be inhibited by chloramphenicol, but not by erythromycin. In contrast, if the extract was not pretreated with chlortetracycline, both chloramphenicol and erythromycin inhibited peptidyl puromycin synthesis. From these observations they proposed that, in the absence of pretreatment with chlortetracycline, newly formed peptidyl tRNA remained in the A-site because of the inhibitory action of erythromycin on translocation to the P-site. With peptidyl tRNA stuck in the A-site, puromycin had no access to the A-site, resulting in inhibition of peptidyl puromycin formation. These early studies of Vazquez (50) and Cundliffe and McQuillen (9) formed the beginnings of the understanding of erythromycin at the molecular level.

***ermC* leader mutations that result in altered specificity of induction.** The *ermC* leader peptide provides a model system for which one can infer more precisely that a critical peptide length for inhibition by erythromycin means beginning with the fourth amino acid residue. Thus, erythromycin does not inhibit peptide bond synthesis per se but, rather, inhibits elongation of peptide chains beyond the third or fourth residue. This inhibition reaction shows selectivity for the next four to six amino acids that, in the case of the amino acid sequence fMGIF SIFVISTV-, is manifested by formation of a stabilized stalled ribosome-mRNA complex.

Vazquez (50) had shown earlier that polyphenylalanine synthesis directed by polyuridylic acid was relatively insusceptible to erythromycin; in a comparison of the relative susceptibilities of polyphenyalanine, polylysine, and polyproline synthesis directed in cell-free reactions by their respective homopolymeric messengers, 0.15 mM erythromycin inhibited these reactions by 12, 57, and 85%, respectively. Odom et al. (40) have sought to explain these findings in terms of extreme conformational differences assumed by these homopolymeric amino acids. They determined that nascent peptides (in the form of peptidyl tRNA) containing more than a few amino acid residues block the binding of erythromycin to ribosomes, with a notable exception of polyphenylalanine. They also correlated these findings with the observation that long polyphenylalanine chains (ca. 100 residues) can be synthesized on ribosomes to which erythromycin is bound. These data suggest a three-way correlation between polypeptide conformation, inhibition of peptide synthesis by erythromycin, and inhibition by the polypeptide of erythromycin binding to the ribosome. In view of the relative efficiency with which genes can be assembled from chemically synthesized DNA fragments, it would be of interest to examine the erythromycin susceptibility of the synthesis of defined complex amino acid sequences selected on the basis of their similarities to known leader peptides.

It is therefore necessary but not sufficient that an antibiotic inhibit protein synthesis for it to induce *ermC* expression; it should also stabilize, rather than destabilize, polysomes, which is not consistent with the well-known effect of erythromycin, which destabilizes this association. Does the stabilization-destabilization of polysomes reflect a new phenomenon, or is it an extension of the inhibition-no inhibition of erythromycin binding vis-à-vis extension of peptidyl tRNA beyond the four-amino-acid length. One could argue that the stabilization of polysomes is associated with the nascent peptidyl chain becoming "stuck" and that this reflects an intermediate state between pure obstruction of peptide chain elongation by erythromycin (characteristic of polylysine synthesis) and "slide-around erythromycin" (characteristic of polyphenylalanine synthesis). The identification of -(FS)IFVI- as the *ermC* amino acid leader sequence that is critical for induction suggests additional model sequences that might be synthesized to determine systematically the range of amino acid sequence that is capable of supporting induction. The dependence of induction specificity, i.e., which amino acid induces as a function of leader peptide amino acid, can be demonstrated experimentally. In a set of leader peptide mutants that were constructed, changes in the relative activities of erythromycin and celesticetin as inducers were noted (35).

The role of the amino acid sequence was examined in greater detail by comparing the effects of amino acid changes in the *ermC* leader peptide on induction by a test panel of three antibiotics (35). The results are summarized in Fig. 2. First, the patterns of inducibility by erythromycin and megalomicin, both 14-membered-ring macrolides, resemble each other more than the pattern of inducibility by each one resembles that by celesticetin. Thus, not all inducing antibiotics induce *ermC* identically; antibiotic structure plays a role in determining whether it will induce in a specific peptide-synthesizing environment. Second, conservative amino acid alterations had marked effects on inducibility. There were five mutations to leucine: I3L, I6L, F7L, V8L, I9L. All of the mutations conserved the hydrophobic character of the amino acid side chain. Two of these, F7L and V8L, reduced induction by erythromycin, while the remainder had no effect. Third, the mutation F4I reduced induction by celesticetin but not that by erythromycin or megalomicin, whereas the mutation S5I reduced induction by either erythromycin or megalomicin but not that by celesticetin. These observations point to a set of complex relationships between ribosome, antibiotic, and leader peptide amino acid sequence and how the variability of induction specificity can be manipulated.

The course of nascent peptide growth in relation to the peptidyl transferase circle. Stade et al. (48) have studied the ribosomal synthesis of the tetrapeptide MKFE by a defined *E.*

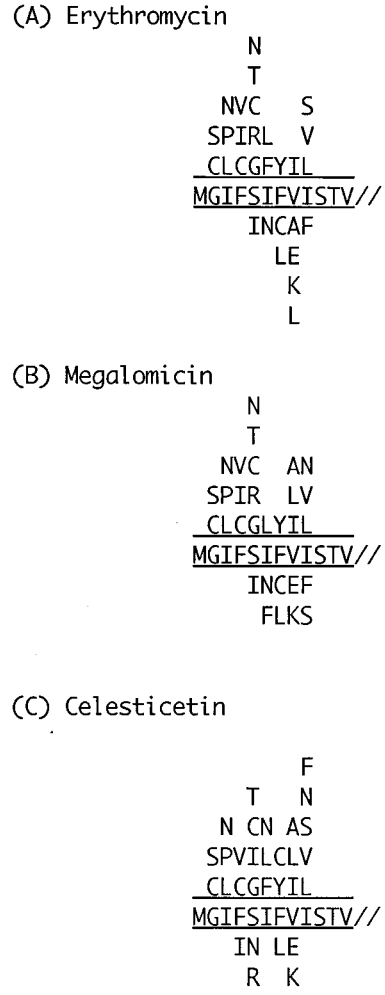

FIG. 2. Effect of altering leader peptide amino acid on efficiency of induction by test antibiotics. The first 12 amino acids of the *ermC* leader peptide are shown together with amino acid alterations in the *ermC* leader peptide sequence, which were obtained by cassette mutagenesis as described previously (36). The same set of mutants was induced with antibiotics shown to have inducing activity: erythromycin (A), megalomicin (B), or (C) celesticetin (C). Induction was quantified by measuring the β-galactosidase activity of LacZ translationally fused to ErmC. Single amino acid changes that reduced induction are entered below their respective locations in the leader peptide sequence; amino acid changes that did not alter induced expression are correspondingly entered above. Data are reported only up to Ile-9, since substitution of a UAA stop codon for the Ile-9 codon AUC abolished induction, whereas the same substitution for ACG, the codon for Ser-10, did not. A total of 14 amino acid substitutions covering Ser-10, Thr-11, and Val-12 showed wild-type induction (data not shown).

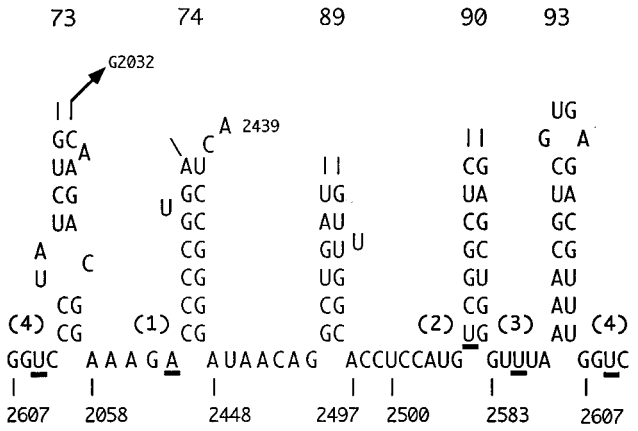

FIG. 3. Peptidyl transferase circle. Linear representation showing sites at which the photoreactive affinity label *N*-α-arylazide Met-tRNA^{fMet} formed cross-links with nucleotides in 23S rRNA as reported by Stade et al. (48). Bases at which cross-links were formed are underlined and are labeled numerically in order of the successive appearance of cross-linkage coincident with the stepwise addition of four amino acids to the nascent peptide chain. The four bases involved are close to bases that either are protected by bound antibiotic or confer resistance as a result of mutation. The mutation A-2062-C, in the first base that forms a cross-link with the photoprobe, confers resistance to chloramphenicol and is strongly protected by carbomycin and streptogramin B. See text for details and Fig. 1 in the previous minireview (56).

coli cell-free model system. The length of peptide synthesized was controlled by the charged aminoacyl tRNAs that were added to the reaction mixture. Met- and Lys-charged tRNAs were converted into photoaffinity probes by the addition of aryldiazirine moieties to the respective α- and ε-amino groups of Met and Lys. By stepwise elongation of the peptide chain and irradiation of the reaction mixture following each round of elongation they were able to form cross-links to specific nucleotides in the 23S rRNA accessible to, and therefore presumably along, the path of growth of the nascent peptide chain. Their studies, summarized in Fig. 3, showed that both the orientation and the extent of the process were defined by the formation of a cross-link between Met and, successively, A-2062, U-2506, U-2585, and U-2609, all of which are located within the peptidyl transferase center. Synthesis of the complete tetrapeptide allowed formation of a more distant cross-link to U1781 located in domain IV.

The observations of Stade et al. (48) put the amino terminus of the nascent peptide on a collision course with erythromycin bound to or in the vicinity of A-2058 and provide support for the obstruction model of erythromycin action (cf. Fig. 1 in a previous minireview [56]). A different (but less informative) pattern of nucleotide derivatization was seen if the ε-Lys derivative was used instead, suggesting that the side chain of the second amino acid points at different nucleotides along the path that it traverses. It will be of interest to see the pattern of cross-linking in an erythromycin-inhibited system. Additional cross-linking data documenting the proximity of the peptidyl transferase circle to other regions in 23S and 5S rRNA have been reported (12, 13) and may eventually be found to have a bearing on the action of erythromycin.

Comparison of *ermC* and *cat-86* attenuators. An intrinsic function for the *ermC* leader peptide has not yet been found, in part, because biochemical studies with it are limited by its low solubility in aqueous solutions. *cat-86* confers inducible resistance to chloramphenicol and is also regulated by a translational attenuator (for a review, see reference 33). The *cat-86* leader peptide has the amino acid sequence MVKTDKISS, and Gu et al. (19–21) have proposed a model according to which the chloramphenicol-induced leader peptide stall product, MVKTD, in turn, inhibits peptidyl transferase activity. Indeed, inhibition of the peptidyl transferase activity of phenol-extracted rRNA by chemically synthesized MVKTD was seen, albeit with a 50% inhibitory concentration of 0.5 to 1 mM, depending on the source of the ribosomes. In support of their model, they noted a correlation between the inhibitory activity of a test peptide and the inducibility of a *cat* attenuator that encodes the same attenuator peptide. A direct interaction between MVKTD and A-2058 and A-2059 of the *B. subtilis* peptidyl transferase circle was also seen in dimethylsulfate protection experiments (20). As discussed above, not all inhibited ribosomes are necessarily stably stalled in a way that will

support induction. Thus, the proposed model may provide additional features that are required to convert inhibition of peptidyl transferase activity by chloramphenicol into a stable ribosome stall signal. It will be of interest to see whether MVKTD acts in vivo as the free peptide or as the peptidyl tRNA complex and whether the reported interaction between MVKTD and the peptidyl transferase circle can also inhibit methylation of A-2058.

A MODEL FOR THE ACTION OF ERYTHROMYCIN

A unified model to explain the diverse effects of 14- and 16-membered-ring macrolides acting on the synthesis of different polypeptides would postulate that (i) macrolide antibiotics bind near the peptidyl transferase center and, depending on the length of the nascent peptide and its conformation, will or will not inhibit further elongation. (ii) Larger (primarily 16-membered-ring) macrolides can block the emergence of the nascent peptide from the ribosome through a virtual (or real) channel; this leads to inhibition of the elongation of most peptides and to destabilization of the ribosome-peptidyl-tRNA complex. Such antibiotics will not induce. (iii) Smaller (primarily 14-membered-ring) macrolides can produce only a partial block of the nascent peptide channel and will inhibit elongation, depending on the conformation of the nascent peptide. A nascent peptide such as oligophenylalanine will slip around the bound erythromycin and no inhibition will be seen, whereas a nascent peptide such as oligolysine or most natural proteins will be completely blocked, leading to destabilization of the ribosome-peptidyl-tRNA complex. (iv) The intermediate case would be the most relevant. The elongation of the nascent peptide chain becomes stuck while transversing the nascent peptide channel. Protein synthesis is inhibited, but the ribosome-peptidyl-tRNA complex is stabilized. Because of the time scale of inhibition relative to that of transcription, this interaction leads to induction. (v) Stabilization of the ribosome-peptidyl-tRNA complex has as its corollary the physical stabilization of the *ermC* or *ermA* message against nucleolytic degradation, providing a barrier against degradation by a $5'\rightarrow3'$ exonuclease (references 4, 11, and 44, respectively).

Apart from the mechanics of induction at the polypeptide level, it is worth considering the possible role of rare codon usage in maximizing the probability that the ribosome will stall at the critical location that encodes -IFVI-. Limitation of aminoacyl tRNA most closely mimics, functionally, the presence of both a rarely used codon together with the relatively low concentration of its cognate aminoacyl tRNA; in both cases protein synthesis is slowed without antibiotic molecules cluttering the ribosome. Kadam (26) has shown that pseudomonic acid, which selectively inhibits Ile-tRNA synthetase, induces *ermC* translationally fused to *lacZ*. The codons that encode Ile in the leader peptide are Ile-3, AUU; Ile-6, AUU; and Ile-9, AUC. Shields and Sharp (45) have compiled data on codon usage in *B. subtilis*. They reported AUC > AUU >> AUA as the order of the relative frequencies with which the respective Ile codons are used in *B. subtilis* protein synthesis. Thus, availability of Ile, whose limitation by pseudomonic acid has been shown to induce *ermC*, is encoded by its two more abundantly used codons.

What distinguishes the inhibitory action of MLS antibiotics that induce *ermC* from those that do not? In vivo footprinting studies of the *ermC* message demonstrated mRNA protection by ribosomes only in the presence of antibiotics shown previously to induce; it failed to show any mRNA protection by noninducing antibiotics (34). The noninducing MLS antibiotics were used at concentrations that inhibit protein synthesis. These findings suggest that MLS antibiotics that fail to induce facilitate polysome breakdown rather than stabilization. It is only when erythromycin inhibits protein synthesis accompanied by pausing at -IFVI- in the *ermC* leader peptide or its functional equivalent in other leader peptides (see below) that mRNA protection, stabilization, and induction are seen. For most amino acid sequences, inhibition by erythromycin produces the opposite effect.

Thus, Tai et al. (49) reported that erythromycin caused the breakdown of polysomes and proposed that under the influence of erythromycin, ribosomes enter a cycle of repetitive abortive initiations. In a similar vein, Menninger (38) and Menninger and Otto (37) noted enhanced in vivo release of peptidyl tRNA in cells treated with erythromycin, spiramycin, or carbomycin. Andersson and Kurland (3) focused their attention on the effect of erythromycin on β-galactosidase synthesis in vivo and concluded that an initial stage of translation is susceptible to erythromycin but that the elongating ribosome is insusceptible. Vester and Garrett (53) likewise observed that erythromycin destabilizes mRNA-bound 70S ribosomes, acting at some stage prior to tetrapeptide formation; however, they reported that the 50S subunits that were released were selectively degraded, which would prevent their recycling.

These observations are consistent with the model of induction discussed above, with the added condition that leader peptides represent an exceptional minority fraction of the total repertoire of sequences. Owing to special properties of the amino acid sequence comprising residues 4 through 9, the association of the ribosome with mRNA is actually stabilized in the presence of erythromycin.

Inhibition of synthesis of an experimental leader peptide that has some arbitrary amino acid sequence per se does not suffice for induction (35). The problem therefore is to determine the range of leader peptide sequences that can support induction and that are therefore functionally equivalent to -IFVI-. The 14-amino-acid leader peptide of *ermD* (17) (also *ermJ* [27] and *ermK* [29]), MTHSMRLRFPTLNQ, has an amino acid sequence that differs from that of the *ermC* leader peptide, and there appears to be no obvious alignment of the two sequences. Likewise for the 36-amino-acid leader peptide of *ermAM* (23) and the leader peptides of inducible actinomycete *erm* genes (56). Thus, we have candidates for amino acid sequences that are functionally equivalent to, but structurally different from, -IFVI-. It is hoped that a systematic pattern will emerge from a comparative examination of these amino acid sequences and that a clue to the basis for the exceptional behavior of ribosomes in the presence of bound erythromycin will be provided.

CONCLUSION

In the wake of early clinical developments in the study of erythromycin-inducible resistance, it was expected that one might use the noninducing MLS antibiotics to treat infectious diseases caused by the erythromycin-inducible strains. Sixteen-membered-ring macrolides—spiramycin, leucomycin, and carbomycin—as well as the lincosamides and streptogramin type B antibiotics were initially considered drugs of choice against erythromycin-resistant staphylococci because they produced clear inhibition zones whose diameters indicated susceptibility in these strains. A practical consequence of understanding the inducible nature of MLS resistance was to explain how a noninducing MLS antibiotic such as spiramycin, pristinamycin, or lincomycin to which the clinical isolate initially appeared to be susceptible could select mutants that are constitutively resistant to all MLS antibiotics. Despite the chemical differences

between the MLS antibiotics, resistant cells that were selected with any one of them became resistant to all of them in an apparent single mutational step.

In the clinical setting this mean that, despite appearances of susceptibility, staphylococci carrying inducible erythromycin resistance could mutate to constitutive expression of resistance directed to all MLS antibiotics with a high probability and with a resultant increased morbidity. In one such case, reported by Watanakunakorn (54), a patient was treated for endocarditis caused by erythromycin (inducibly)-resistant *S. aureus*. The strain was reported to be susceptible to clindamycin, and the patient responded favorably to clindamycin treatment for 26 days, but this was followed by a relapse associated with the development of constitutive resistance in the infecting strain.

Attempts have therefore been made to synthesize macrolides that will inhibit ribosomes in which A-2058 has been methylated. Goldman and Kadam (15) reported on an 11-carbamino-6-*O*-methyl derivative of erythromycin that can bind to resistant ribosomes and that can inhibit protein synthesis. The respective 50% inhibitory doses of erythromycin and this derivative for the inhibition of protein synthesis in vitro were 0.4 and 20 μM, respectively. The derivative did not induce either *ermC* or *ermD*. It will be interesting to see which nucleotides in 23S rRNA are protected by this group of antibiotics and whether useful drugs will be developed from this lead.

In summary, inducibly resistant bacteria have discovered a class of exceptional peptide sequences whose synthesis leads to a violation of the established rules of engagement between erythromycin and the ribosome. According to these rules, erythromycin is supposed to destabilize the association between peptidyl tRNA, mRNA, and the ribosome, and thereby derail the protein-synthetic machinery. During the synthesis of one of these exceptional sequences in the presence of erythromycin, the ternary association between ribosome, mRNA, and nascent protein actually becomes stabilized. By the devious placement of this sequence at a critical location in a leader peptide, bacteria have evolved a mechanism to control mRNA conformation and thereby to regulate the expression of erythromycin resistance.

ACKNOWLEDGMENTS

I am grateful to J. Davies and H. Kirst as well to an anonymous referee who made numerous helpful suggestions for the improvement of the manuscript. I also thank C. Dizak and R. Giannattasio for expertise in preparing the figures.

This work was supported by NIH grant AI-18283.

REFERENCES

1. **Allen, N. E.** 1977. Macrolide resistance in *Staphylococcus aureus*: induction of macrolide resistant protein synthesis. Antimicrob. Agents Chemother. **11:** 661–668.
2. **Allen, N. E.** 1977. Macrolide resistance in *Staphylococcus aureus*: inducers of macrolide resistance. Antimicrob. Agents Chemother. **11:**669–674.
3. **Andersson, S., and C. G. Kurland.** 1987. Elongating ribosomes in vivo are refractory to erythromycin. Biochimie **69:**901–904.
4. **Bechhofer, D. H., and K. H. Zen.** 1989. Mechanism of erythromycin-induced *ermC* mRNA stability in *Bacillus subtilis*. J. Bacteriol. **171:**5803–5811.
5. **Bourse, R., and J. Monier.** 1967. Effet de l'érythromycine sur la croissance de *Staph. aureus* "resistant dissocié" en bactériostase par un autre macrolide ou un antibiotique apparenté. Ann. Inst. Pasteur (Paris) **113:**67–79.
6. **Breidt, F., and D. Dubnau.** 1990. Identification of *cis*-acting sequences required for translational autoregulation of the *ermC* methylase. J Bacteriol. **172:**3661–3668.
7. **Chabbert, Y.** 1956. Antagonisme in vitro entre l'erythromycine et la spiramycine. Ann. Inst. Pasteur (Paris) **90:**787–790.
8. **Contreras, A., and D. Vazquez.** 1977. Cooperative and antagonistic interactions of peptidyl-tRNA and antibiotics with bacterial ribosomes. Eur. J. Biochem. **74:**539–547.
9. **Cundliffe, E., and K. McQuillen.** 1967. Bacterial protein synthesis: the effects of antibiotics. J. Mol. Biol. **30:**137–146.
10. **Denoya, C., D. H. Bechhofer, and D. Dubnau.** 1986. Translational autoregulation of *ermC* 23S rRNA methyltransferase expression in *Bacillus subtilis*. J. Bacteriol. **168:**1133–1141.
11. **DiMari, J. F., and D. H. Bechhofer.** 1993. Initiation of mRNA decay in *Bacillus subtilis*. Mol. Microbiol. **7:**705–717.
12. **Dontsova, O., V. Tishkov, S. Dokudovskaya, A. Bogdanov, T. Döring, J. Rinke-Appel, S. Thamm, B. Greuer, and R. Brimacombe.** 1994. Stem-loop IV of 5S rRNA lies close to the peptidyltransferase center. Proc. Natl. Acad. Sci. USA **91:**4125–4129.
13. **Döring, T., B. Greuer, and R. Brimacombe.** 1991. The three-dimensional folding of ribosomal RNA; localization of a series of intra-RNA cross-links in 23S RNA induced by treatment of *Escherichia coli* 50S ribosomal subunits with bis-(2-chloroethyl)-methylamine. Nucleic Acids Res. **19:**3517–3524.
14. **Dubnau, D.** 1985. Induction of *ermC* requires translation of the leader peptide. EMBO J. **4:**533–537.
15. **Goldman, R. C., and S. K. Kadam.** 1989. Binding of novel macrolide structures to macrolides-lincosamides-streptogramin B-resistant ribosomes inhibits protein synthesis and bacterial growth. Antimicrob. Agents Chemother. **33:**1058–1066.
16. **Gryczan, T., G. Grandi, J. Hahn, R. Grandi, and D. Dubnau.** 1980. Conformational alteration of mRNA structure and the posttranscriptional regulation of erythromycin-induced drug resistance. Nucleic Acids Res. **8:**6081–6097.
17. **Gryczan, T., M. Israeli-Reches, M. Del Bue, and D. Dubnau.** 1984. DNA sequence and regulation of *ermD*, a macrolide-lincosamide-streptogramin B resistance element from *Bacillus licheniformis*. Mol. Gen. Genet. **194:**349–356.
18. **Gryczan, T. J., M. Israeli-Reches, and D. Dubnau.** 1984. Induction of macrolide-lincosamide-streptogramin B resistance requires ribosomes able to bind inducer. Mol. Gen. Genet. **194:**357–361.
19. **Gu, Z., R. Harrod, E. J. Rogers, and P. S. Lovett.** 1994. Anti-peptidyltransferase leader peptides of attenuation-regulated chloramphenicol-resistance genes. Proc. Natl. Acad. Sci. USA **91:**5612–5616.
20. **Gu, Z., R. Harrod, E. J. Rogers, and P. S. Lovett.** 1994. Properties of a pentapeptide inhibitor of peptidyltransferase that is essential for *cat* gene regulation by translational attenuation. J. Bacteriol. **176:**6238–6244.
21. **Gu, Z., E. J. Rogers, and P. S. Lovett.** 1993. Peptidyl transferase inhibition by the nascent leader peptide of an inducible *cat* gene. J. Bacteriol. **175:**5309–5313.
22. **Hahn, J., G. Grandi, T. J. Gryczan, and D. Dubnau.** 1982. Translational attenuation of *ermC*: a deletion analysis. Mol. Gen. Genet. **186:**204–216.
23. **Horinouchi, S., W.-H. Byeon, and B. Weisblum.** 1983. A complex attenuator regulates inducible resistance to macrolides, lincosamides, and streptogramin type B antibiotics in *Streptococcus sanguis*. J. Bacteriol. **154:**1252–1262.
24. **Horinouchi, S., and B. Weisblum.** 1980. Posttranscriptional modification of mRNA conformation: mechanism that regulates erythromycin-induced resistance. Proc. Natl. Acad. Sci. USA **77:**7079–7083.
25. **Horinouchi, S., and B. Weisblum.** 1981. The control region for erythromycin resistance: free energy changes related to induction and mutation to constitutive expression. Mol. Gen. Genet. **182:**341–348.
26. **Kadam, S. K.** 1989. Induction of *ermC* methylase in the absence of macrolide antibiotics and by pseudomonic acid A. J. Bacteriol. **171:**4518–4520.
27. **Kim, H.-S., E.-C. Choi, and B.-K. Kim.** 1993. A macrolide-lincosamide-streptogramin B resistance determinant from *Bacillus anthracis* 590: cloning and expression of *ermJ*. **139:**601–607.
28. **Kolter, R., and C. Yanofsky.** 1982. Attenuation in amino acid biosynthesis operons. Annu. Rev. Genet. **16:**113–134.
29. **Kwak, J.-H., E.-C. Choi, and B. Weisblum.** 1991. Transcriptional attenuation control of *ermK*, a macrolide-lincosamide-streptogramin B resistance determinant from *Bacillus licheniformis*. J. Bacteriol. **173:**4725–4735.
30. **Lai, C.-J., J. E. Dahlberg, and B. Weisblum.** 1973. Structure of an inducibly methylatable nucleotide sequence in 23S ribosomal ribonucleic acid from erythromycin-resistant *Staphylococcus aureus*. Biochemistry **12:**457–460.
31. **Lai, C.-J., and B. Weisblum.** 1971. Altered methylation of ribosomal RNA in an erythromycin-resistant strain of *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **68:**856–860.
32. **Lampson, B. C., and J. T. Parisi.** 1986. Naturally occurring *Staphylococcus epidermidis* plasmid expressing constitutive macrolide-lincosamide-streptogramin B resistance contains a deleted attenuator. J. Bacteriol. **166:**479–483.
33. **Lovett, P. S.** 1990. Translational attenuation as the regulator of inducible *cat* genes. J. Bacteriol. **172:**1–6.
34. **Mayford, M., and B. Weisblum.** 1989. Conformational alterations in the *ermC* transcript in vivo during induction. EMBO J. **8:**4307–4314.
35. **Mayford, M., and B. Weisblum.** 1989. *ermC* leader peptide, amino acid sequence critical for induction by translational attenuation. J. Mol. Biol. **206:**69–79.
36. **Mayford, M., and B. Weisblum.** 1990. The *ermC* leader peptide: amino acid alterations leading to differential efficiency of induction by macrolide-lincosamide-streptogramin B antibiotics. J. Bacteriol. **172:**3772–3779.
37. **Menninger, J. R.** 1985. Functional consequences of binding macrolides to

ribosomes. J. Antimicrob. Chemother. **16**(Suppl. A)**:**23–34.
38. **Menninger, J. R., and D. P. Otto.** 1982. Erythromycin, carbomycin, and spiramycin, inhibit protein synthesis by stimulating the dissociation of peptidyl-tRNA from ribosomes. Antimicrob. Agents Chemother. **21:**811–818.
39. **Narayanan, C. S., and D. Dubnau.** 1987. Demonstration of erythromycin-dependent stalling of ribosomes on the *ermC* leader transcript. J. Biol. Chem. **262:**1766–1771.
40. **Odom, O. W., W. D. Picking, T. Tsalkova, and B. Hardesty.** 1991. The synthesis of polyphenylalanine on ribosomes to which erythromycin is bound. Eur. J. Biochem. **198:**713–722.
41. **Pattee, P. A., and J. N. Baldwin.** 1962. Transduction of resistance to some macrolide antibiotics in *Staphylococcus aureus*. J. Bacteriol. **84:**1049–1055.
42. **Pestka, S., R. Vince, R. LeMahieu, F. Weiss, L. Fern, and J. Unowsky.** 1976. Induction of erythromycin resistance in *Staphylococcus aureus* by erythromycin derivatives. Antimicrob. Agents Chemother. **9:**128–130.
43. **Ryabova, L. A., O. M. Selivanova, V. I. Baranov, V. D. Vasiliev, and A. S. Spirin.** 1988. Does the channel for nascent peptide exist inside the ribosome? Immune electron microscopy study. FEBS Lett. **226:**255–260.
44. **Sandler, P., and B. Weisblum.** 1989. Erythromycin-induced ribosome stall in the *ermA* leader: a barricade to 5′-to-3′ nucleolytic cleavage of the *ermA* transcript. J. Bacteriol. **171:**6680–6688.
45. **Shields, D. C., and P. M. Sharp.** 1987. Synonymous codon usage in *Bacillus subtilis* reflects both translational selection and mutational biases. Nucleic Acids Res. **15:**8023–8040.
46. **Shivakumar, A. G., J. Hahn, G. Grandi, Y. Kozlov, and D. Dubnau.** 1980. Posttranscriptional regulation of an erythromycin resistance protein specified by plasmid pE194. Proc. Natl. Acad. Sci. USA **77:**3903–3907.
47. **Skinner, R., E. Cundliffe, and F. J. Schmidt.** 1983. Site of action of a ribosomal RNA methylase responsible for resistance to erythromycin and other antibiotics. J. Biol. Chem. **258:**12702–12706.
48. **Stade, K., S. Riens, D. Bochkariov, and R. Brimacombe.** 1994. Contacts between the growing peptide chain and the 23S RNA in the 50S ribosomal subunit. Nucleic Acids Res. **22:**1394–1399.
49. **Tai, P.-C., B. J. Wallace, and B. D. Davis.** 1974. Selective action of erythromycin on initiating ribosomes. Biochemistry **13:**4653–4659.
50. **Vazquez, D.** 1966. Binding of chloramphenicol to ribosomes. The effect of a number of antibiotics. Biochim. Biophys. Acta **114:**277–288.
51. **Vazquez, D.** 1966. Antibiotics affecting chloramphenicol uptake by bacteria. Their effect on amino acid incorporation in a cell-free system. Biochim. Biophys. Acta **114:**289–295.

51a. **Vazquez, D.** 1979. Inhibitors of protein biosynthesis. Mol. Biol. Biochem. Biophys. **30:**1–312.

52. **Vazquez, D., and R. E. Munro.** 1967. Effects of some inhibitors of protein synthesis on the binding of aminoacyl tRNA to ribosomal subunits. Biochim. Biophys. Acta **142:**155–173.
53. **Vester, B., and R. A. Garrett.** 1987. A plasmid-coded and site-directed mutation in *Escherichia coli* 23S RNA that confers resistance to erythromycin: implications for the mechanism of action of erythromycin. Biochimie **69:**891–900.
54. **Watanakunakorn, C.** 1976. Clindamycin therapy of *Staphylococcus aureus* endocarditis. Clinical relapse and development of resistance to clindamycin, lincomycin, and erythromycin. Am. J. Med. **60:**419–425.
55. **Weaver, J. R., and P. A. Pattee.** 1964. Inducible resistance to erythromycin in *Staphylococcus aureus*. J. Bacteriol. **88:**574–580.
56. **Weisblum, B.** 1995. Erythromycin resistance by receptor modification. Antimicrob. Agents Chemother. **39:**577–585.
57. **Weisblum, B., and V. Demohn.** 1969. Erythromycin-inducible resistance in *Staphylococcus aureus*: survey of antibiotic classes involved. J. Bacteriol. **98:**447–452.
58. **Weisblum, B., C. Siddhikohl, C.-J. Lai, and V. Demohn.** 1971. Erythromycin-inducible resistance in *Staphylococcus aureus*: requirements for induction. J. Bacteriol. **106:**835–847.
59. **Yonath, A., K. R. Leonard, and H. G. Wittmann.** 1987. A tunnel in the large ribosomal subunit revealed by three-dimensional image reconstruction. Science **236:**813–816.