Volume 8 Number 24 1980 Nucleic Acids Research

Conformational alteration of mRNA structure and the posttranscriptional regulation of erythromycin-induced drug resistance

T.J.Gryczan, G.Grandi, J.Hahn, R.Grandi and D.Dubnau

Department of Microbiology, The Public Health Research Institute of The City of New York, Inc., 455 First Avenue, New York, NY 10016, USA

Received 26 September 1980

ABSTRACT

The DNA sequence of the ermC gene of plasmid pE194 is presented. This determinant is responsible for erythromycin-induced resistance to the macrolide-lincosamide-streptogramin B group of antibiotics and specifies a 29,000 dalton inducible protein. The locations of the ermC promoter, as well as that of a probable transcriptional terminator, are established both from the sequence and by transcription mapping. The sequence contains an open reading frame sufficient to encode the previously identified 29,000 dalton ermC protein. Between the promoter and the putative ATG start codon is a 141 base pair leader sequence, within which several regulatory (constitutive) mutations have been mapped and sequenced. The leader has a second open reading frame, sufficient to encode a 19 amino acid peptide. It is suggested that induction by erythromycin involves a shift between alternative ribosome-bound mRNA conformations, so that the ribosome binding sequence and the start codon for synthesis of the 29K protein are unmasked in the presence of inducer. Possible active and inactive folded configurations of the leader sequence are presented, as well as the effects on these configurations of regulatory mutations.

INTRODUCTION

Plasmid-mediated resistance to the clinically important macrolide-lincosamide-streptogramin B (MLS) group of antibiotics in all cases studied to date, is associated with a specific dimethylation of adenine in the 23S RNA of the large ribosomal subunit (1-3). pE194, a 3.5 kilobase multicopy plasmid derived from Staphylococcus aureus (4) and introduced by transformation into Bacillus subtilis (5), carries the ermC determinant which confers resistance to MLS antibiotics. This resistance is inducible by subinhibitory concentrations of erythromycin (Em) (5), as is a 29,000 dalton polypeptide specified by ermC (6). Plasmid mutants (tyc) which confer resistance constitutively, are also constitutive for the expression of the 29K protein (6). The ermC gene, together with sequences carrying the information required for inducibility, have been localized on the physical map of pE194, and the direction of transcription has been determined (7). Recently we

have reported that induction of the 29K protein and of MLS-resistance by Em is regulated posttranscriptionally, and that interaction of Em with a binding site on an unmethylated ribosome is required for regulation (8). Since the 29K protein is required for the expression of MLS-resistance and since the induction of resistance and of 29K protein synthesis are concomitant and affected similarly by tyc mutations, it is reasonable to assume that this protein is a ribosomal RNA (rRNA) methylase.

In this report we present further data, including DNA sequence information, which supports the posttranscriptional nature of 29K protein regulation and which suggests a more specific model of regulation at the level of translation.

MATERIALS AND METHODS

Plasmid and strains

The plasmid used for most procedures including sequencing was pBD15, a high copy number (cop-6) derivative of pE194 (5). Three spontaneous tyc mutants of pE194, which express MLS-resistance constitutively, were also used; pBD16 (tyc-1), pBD73 (tyc-9), and pBD74 (tyc-16). The strains used for plasmid preparations and for isolation of RNA were all trpC2 thr-5.

Isolation of plasmid DNA

Plasmid DNA was isolated and purified from stationary phase cultures grown at 32-34° using CsCl-ethidium bromide (EtBr) density gradient centrifugation as described (9) except that two cycles of CsCl-EtBr centrifugation were used.

DNA sequencing

The procedures of Maxam and Gilbert (10) were used. DNA fragments were end-labeled with ^{32}P-γ-ATP (prepared as described [10]) and then secondarily cleaved with an appropriate restriction endonuclease (Fig. 1). One fragment was also sequenced by strand separation (Fig. 1). Restriction endonucleases were obtained from Bethesda Research Laboratories, New England Biolabs, and Boehringer-Mannheim. Bacterial alkaline phosphatase and polynucleotide kinase were from Bethesda Research Laboratories or New England Biolabs. The A>C, G, C+T, and C reactions were used. All sequences were determined on both strands with the minor exceptions noted in Fig. 2. Cleavage sites used for preparation of fragments were traversed by sequencing overlapping fragments. Thin (0.4 mm) 6, 8 and 20% polyacrylamide-urea gels were used (11).

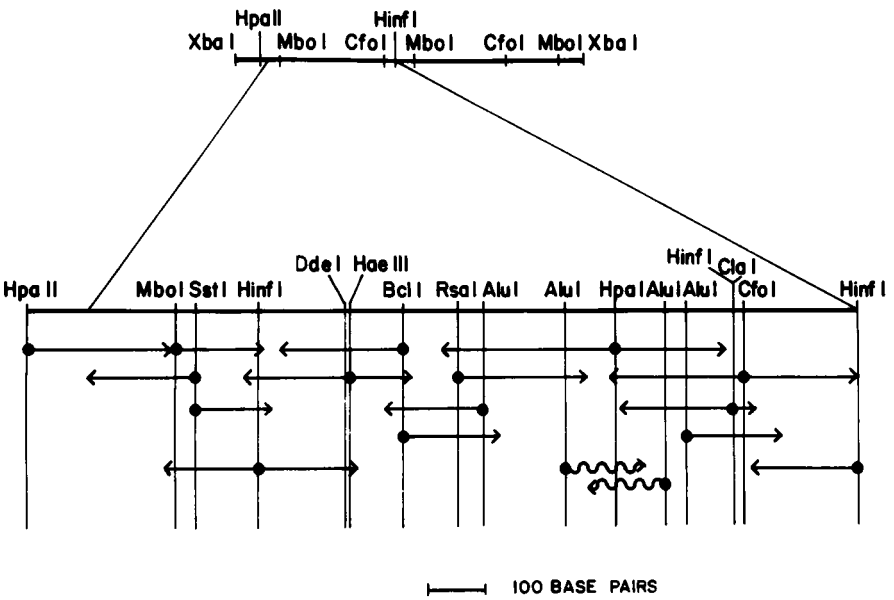


FIG. 1. Sequencing strategy. On the top line is a restriction endonuclease cleavage site map of pET94 (∿3.5 kb). The portion of the plasmid sequenced in this study is shown in expanded form, together with the cleavage sites used. The data illustrate the points of 5' end labeling employed. The arrows show the directions and approximate extents of sequencing in each experiment. All sequencing was by secondary cleavage, except that strand separation was carried out where denoted by wavy lines.

Nick translation

Plasmid DNA was labeled by nick translation to a specific activity of 2×10^8 cpm/μg using ^{32}P-α-ATP (New England Nuclear), as described by Rigby *et al.* (12), but using the DNA polymerase buffer of Maniatis *et al.* (13). DNA polymerase I was from Boehringer Mannheim. Restriction fragments for nick translation were isolated from macerated 7.5% polyacrylamide gel slices, by diffusion (10).

RNA isolation

RNA was prepared from cultures grown to Klett 100 (54 filter) in VY broth (9). The cells were collected by centrifugation and washed twice with RNA-buffer (100 mM NaCl, 10 mM Na-acetate, pH 5.0) containing 1 mM aurintricarboxylic acid. The cells were ruptured as described (8) and the lysates were extracted twice with RNA-buffer-saturated phenol. The RNA was precipitated with two volumes of ethanol and the pellets were rinsed with acetone.

The RNA was redissolved in 0.3 M NH_4-acetate, 0.5% sodium dodecyl sulfate (SDS) and reprecipitated with ethanol. The pellets were rinsed with 70% ethanol, dried, redissolved in TE (10 mM Tris·HCl, pH 7.5, 1 mM EDTA) and stored at -70°.

Immediately before loading on gels the RNA was treated with 0.1 mg/ml RNase-free DNase at 37° for 30 min in the presence of 5 mM $MgCl_2$. Electrophoretically pure DNase (Worthington) was made RNase-free by passage through a column of agarose-5'-(4-aminophenylphosphoryl) uridine-2'(3')phosphate (Miles) (1 ml bed volume) as described (14). The DNase reaction was stopped by the addition of 0.5% SDS and by extracting twice with TE-saturated phenol. The RNA was then precipitated twice with ethanol. The final pellets were dried, redissolved in 10 mM Na phosphate (pH 7.0), 50% dimethylsulfoxide (v/v), 1 M glyoxal, and then incubated at 50° for 1 hr (15).

RNA gels, blotting and hybridization

The RNA samples were loaded onto 1.5% agarose gels in 10 mM Na phosphate, pH 7.0 and run as described by Carmichael and McMaster (15). Blotting of the gels, preparation of diazobenzyloxymethyl (DBM) paper and hybridization was as described by Alwine et al.(16).

A modified Berk-Sharp (17, 18) experiment was carried out using RNA from a strain carrying pBD15. This RNA (25 µg) was mixed with 0.1 µg of HinfI B fragment, 5'-end labeled with ^{32}P-γ-ATP and polynucleotide kinase. The mixture was evaporated to dryness and dissolved in 10 µl of 40 mM Pipes (pH 6.4), 400 mM NaCl, 1 mM EDTA, 80% deionized formamide (v/v). After incubation at 80° for 10 min the mixture was annealed for 4 hr at 35°. These conditions were selected to favor RNA:DNA hybridization of these extremely AT (AU) rich nucleic acid molecules (Fig. 2). To the solution was added 0.3 ml of 280 mM NaCl, 30 mM Na-acetate (pH 4.4), 4.5 mM Zn-acetate, 20 µg/ml denatured salmon sperm DNA, 200 u/ml S1 nuclease. Incubation was continued for 45 min at 30°. The reaction was stopped by the addition of 75 µl 2.5 M NH_4-acetate, 50 mM EDTA. The material was recovered by precipitation with 0.4 ml ethanol and 2 µl E. coli tRNA (10 mg/ml). The samples were loaded on denaturing (12% polyacrylamide-7M urea) or nondenaturing (7.5% polyacrylamide) gels. The running conditions and buffers were as described (19, 20).

RESULTS

Location and properties of the ermC gene

The major ermC gene product is an Em-inducible 29,000 dalton polypeptide (29K protein) which is required for MLS-resistance (6). ermC is located

within the expanded portion of the restriction site map shown in Fig. 1, and is transcribed from left to right on the map (7).

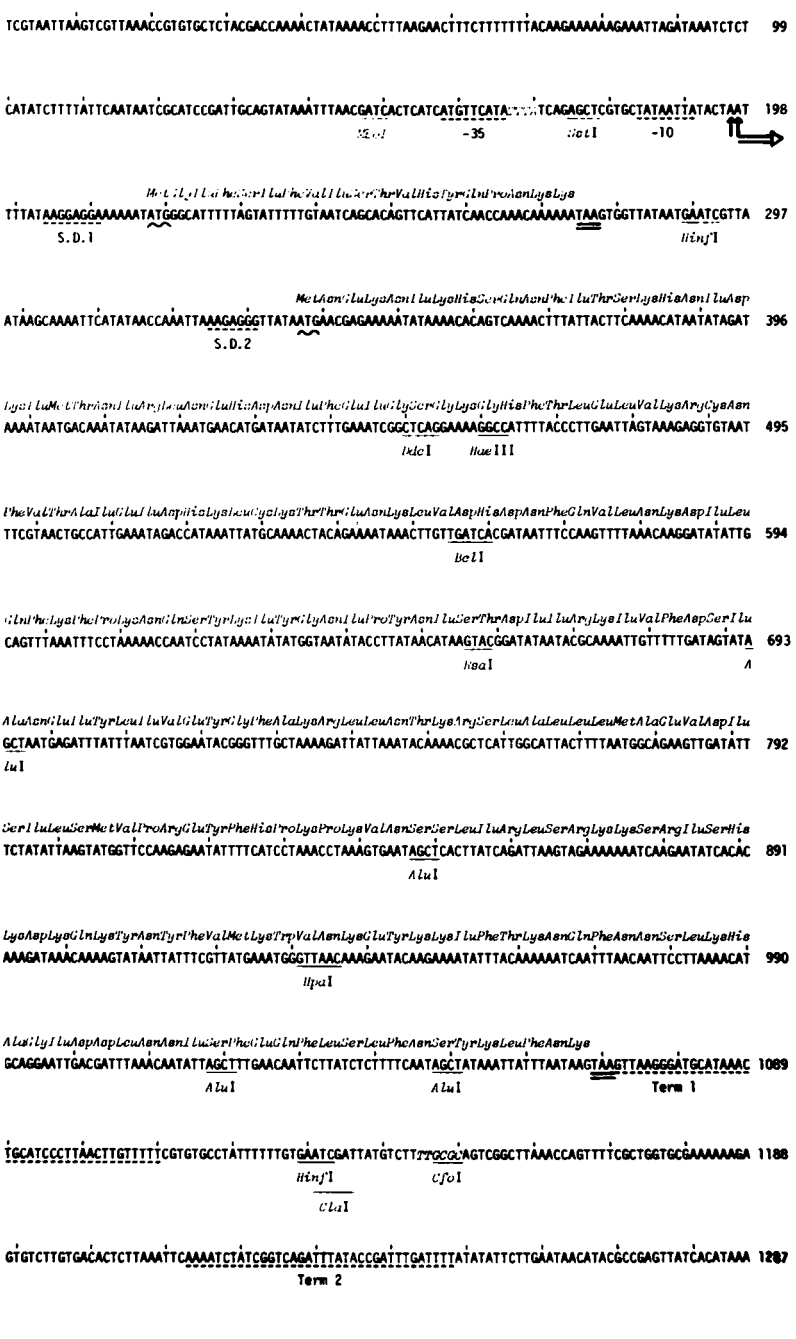
Sequence of ermC

Fig. 2 shows the results of DNA sequencing studies on the region of the pE194 genome containing ermC, in which 1354 base pairs (bp) were determined. The coding strand is shown. Examination of the sequence reveals the following noteworthy features. (1) An open reading frame extends from an ATG triplet at position 337 to a TAA termination codon at position 1069. The alternative frames are replete with UAA, UAG and UGA signals. The open frame suffices to define a protein with 244 amino acids and with a molecular weight of 28,947 daltons, in excellent agreement with our published estimate of 29K for the ermC product (6). Insertion of a chloramphenicol resistance-conferring TaqI fragment from plasmid pC194, into the ClaI cleavage site between positions 1132 and 1133 has no effect on the expression of MLS-resistance (not shown). Insertion of foreign DNA into the BclI site between positions 555 and 556 or deletion at the HpaI site (933-934), inactivates resistance and alters the 29K protein (7, 20). We have observed during purification and from the use of isoelectric-focusing that the 29K protein is basic. This is consistent with examination of the inferred sequence shown in Fig. 2. There are 39 basic residues and 23 acidic ones, with a net positive charge of 16. These observations serve to confirm the assignment of the ermC coding sequence.

Between positions 218 and 274 is an open reading frame (different from the one just noted) which encodes a potential peptide of 19 amino acids and which ends in two lys residues, followed by a TAA signal.

(2) At positions 184-189, 150 base pairs upstream from the probable ATG start codon for the 29K protein, is a TATAAT sequence. This and the surrounding sequences agree well with the consensus "-10" sequence described by Rosenberg and Court (21) as typical of prokaryotic promoters. At positions 158-166 is the sequence ATGTTCATA which bears a slight resemblance to the "-35" consensus sequence TTGACA (21). At positions 196 and 197, appropriately located 7 and 8 bases downstream from the TATAAT sequence, are a pair of A residues which are likely transcriptional start sites. Our previous finding that an RNA polymerase binding site is located very close to the SstI cleavage site between positions 178 and 179 supports the interpretation that this region represents the ermC promoter (7). We present further evidence on this point below.

(3) Several bases upstream from the putative ATG start codon for the 29K



protein is a likely ribosomal binding site (Shine-Dalgarno sequence) (22). This sequence shows excellent complementarity (about a 9 base match) with the sequence at the 3' terminus of 16S rRNA from B. stearothermophilus and probably from B. subtilis as well: 5'-GAUCACCUCCUUUCU(A)-3' (23,24,25). (The underlined residues in this sequence are complementary with the putative Shine-Dalgarno [SD] sequence at positions 325-332.) The spacing between this SD sequence, which we will call SD2, and the ATG start codon, is consistent with observations from other systems (26). The coding sequence for the potential 19 amino acid peptide is preceded by SD1 (positions 204-211), which possesses a 7 base complementarity with the 3' terminal sequence of 16S rRNA.

(4) Two possible transcriptional termination sequences are located within positions 1072-1110 and 1213-1248. Both include inverted repeats, and 19 and 15 bases downstream from the center of the dyad, each contains a run of T's (6 and 4 T's, respectively). This is characteristic of several transcriptional terminators which have been sequenced (21). We believe that the terminator at 1213-1248 (Term 2) is more likely used in vivo (see below).

Transcriptional mapping of ermC

Although the given interpretation of transcriptional signals is reasonable, we considered it essential to buttress these interpretations with direct experimental data. This is particularly important since there is a paucity of sequence data from gram-positive prokaryotes. In at least one case the sequence of an "early" promoter on B. subtilis phage SPO2 has been determined with results consistent with the promoter consensus sequences observed in Escherichia coli (27). On the other hand, B. subtilis is reported to possess forms of RNA polymerase with variant promoter specificities (28) and it seemed prudent to obtain transcriptional mapping data to supplement the sequence analysis.

Fig. 3 presents the results of blotting-hybridization experiments. The ermC transcript was identified by hybridization with a nick translated HaeIII-CfoI fragment which extended from position 462 to 1149. The transcript so identified consists of about 965 bases, which, together with the

FIG. 2. DNA sequence of ermC. The coding strand is shown. All portions were sequenced on both strands, except for the bases in italics, which were determined only on the given strand. The arrows indicate probable sites of transcriptional initiation and the direction of transcription. "-10" and "-35" refer to inferred RNA polymerase binding and recognition elements. Start codons are denoted by wavy lines, and translational stop signals by double lines. "SD" and "Term" refer to Shine-Dalgarno and transcriptional termination signals.

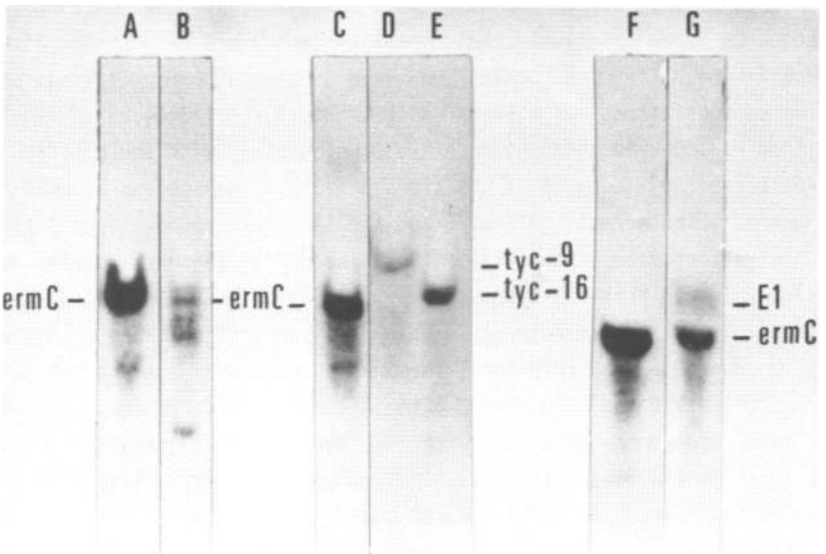


FIG. 3. Blotting-hybridization of the ermC transcript. RNA from a strain carrying pBD15 (A,B,C,F and G), the tyc-9 mutant (D) or the tyc-16 mutant (E) was electrophoresed on agarose gels. The RNA was treated with glyoxal except for F and G. The RNA was blotted onto DBM paper and hybridized with ^{32}P-nick translated probes as follows: A,C,D and E with a CfoI-HaeIII fragment which contains the ermC gene; B with the CfoI B fragment which extends to the right of the region sequenced (see Fig. 1); F with the HaeIII-SstI fragment (Fig. 1); and G with HinfI B, which extends to the left of the region sequenced.

proposed start site at 196-197, suggests that termination occurs near position 1162. A second probe was prepared from the CfoI B fragment of pE194, which extends downstream from position 1149 and continues well beyond the region we have sequenced (Figs. 1,2). It can be seen from Fig. 3B that this probe shows homology with the ermC transcript as well as with several others. This suggests that ermC transcription terminates beyond the CfoI site. The location of the putative Term 2 signal noted above is consistent with this hybridization data.

Three different probes were used to map the start point of ermC transcription. A probe was prepared from a SstI-HaeIII fragment (positions 179-461) and another from the HinfI B fragment. The latter extends upstream from position 289 and includes DNA which we have not sequenced, and which encodes protein E1 (7). Fig. 3 shows that both of these probes hybridize

with the ermC transcript and that the HinfI B probe also has homology with the larger E1 transcript. A third probe was prepared from the MboI fragment which extends upstream from position 150. This probe did not hybridize with the ermC transcript, although it did with the E1 transcript (not shown). These experiments localize the start site for ermC transcription between the MboI site at position 150 and the HinfI site at 289.

To determine more precisely where ermC transcription initiates, we have used a version of the method devised by Berk and Sharp (17, 18). The MboI-HinfI B fragment (positions 150-289) was prepared and labeled at its 5' ends with polynucleotide kinase and ^{32}P-γ-ATP. An attempt to separate the strands of this fragment failed, possibly due to its high A+T content. Instead, the labeled DNA was denatured and annealed to total RNA from a strain carrying pBD15. A portion of the labeled DNA was self-annealed. Both samples were halved and one portion of each was treated with S1 nuclease. The four samples were divided further and loaded on denaturing and nondenaturing polyacrylamide gels, and electrophoresed together with an end-labeled HinfI digest of pBR322 to provide size markers (29) (Fig. 4). Treatment with S1 nuclease

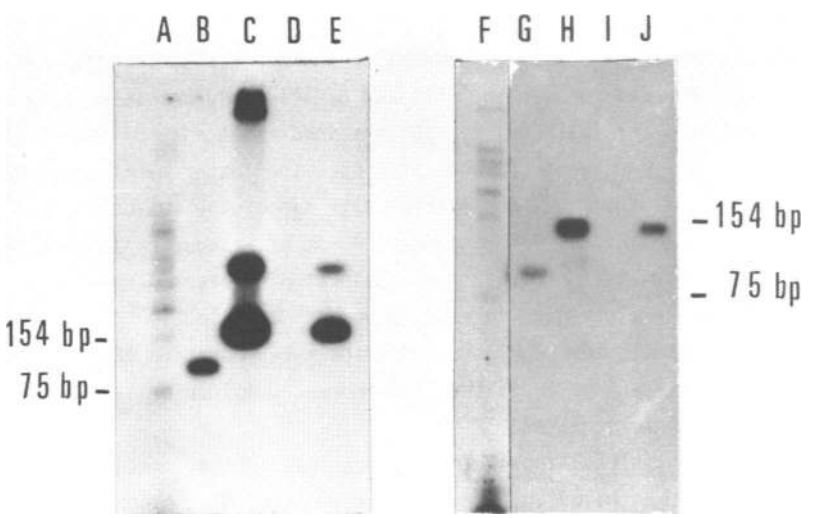


FIG. 4. S1-nuclease transcription mapping of ermC. RNA from a strain carrying pBD15 was annealed to 5' end labeled MboI-HinfI fragment (B,C,G,H). A portion of the DNA was self annealed (D,E,I,J). After hybridization, half of each sample was treated with S1 nuclease (B,D,G,I), and the other half not treated (C,E,H,J). The samples were analyzed on nondenaturing (A-E) and denaturing (F-J) gels. A and F show 5' end labeled HinfI fragments of pBR322 as size markers.

resulted in a total loss of radioactivity from the self-annealed DNA sample, indicating that the conditions used did not favor DNA:DNA renaturation. Treatment of the DNA + RNA sample resulted in the appearance of a single band which consisted of 95 bases (determined from the denaturing gel). Since the direction of transcription is known, an *in vivo* initiation site must be located close to positions 196-197, precisely where we inferred the transcriptional start to be.

The locations of the *ermC* transcriptional and translational starts define a mRNA leader sequence which is 141 bases long.

Regulatory mutants

Selection for the ability of strains carrying pE194 to grow on tylosin (Ty), a noninducing macrolide antibiotic, permits the ready isolation of mutants (*tyc*) which express MLS-resistance without induction (5). Twenty-one spontaneous *tyc* mutants have been isolated and studied. All were plasmid mutants, since transformation of plasmid-free recipients with pure plasmid DNA revealed 100% linkage of the Tyc phenotype with the selected Em resistance. Circular covalently closed (CCC) DNA from the *tyc* mutants was examined on agarose gels. Out of the 21 CCC DNA species studied, twelve were clearly of greater molecular weight than wild type pE194 (3.5 kb). Restriction endonuclease cleavage site mapping of several of these revealed that the extra DNA was always located between the *Sst*I and *Hae*III cleavage sites at positions 179 and 461. For instance, *tyc-16* contained about a 100 bp insert within this segment and *tyc-9* about 650-700 extra bp. The latter mutation is particularly interesting since a restriction site map of the mutant plasmid suggests that it contains a direct tandem repeat of a sequence immediately downstream from the point of insertion. Selection of plasmid revertants with inducible phenotype always led to restoration of the wild type molecular weight. This supports the conclusion that the *tyc-9* mutation results from a direct tandem repeat and shows that the increased molecular size is the cause of the regulatory defect.

Three of the *tyc* mutations which did not result in a detectably increased molecular weight of any fragment derived from digestion by restriction endonucleases (including double digestion by *Hae*III and *Sst*I) were mapped by marker rescue transformation (30). These, including *tyc-1*, were all found to be closely linked to the *Hae*III site.

The RNA from strains carrying the *tyc-9* and *tyc-16* mutant plasmids was analyzed by a blotting-hybridization experiment (Fig. 3C). Both *ermC* transcripts were larger than that of the wild type plasmid (965 bases). The *tyc-16*

transcript contained about 1,050 bases, and the tyc-9 about 1570. Thus, the inserted DNA (about 85 and 600 bp, respectively) was inserted downstream from the ermC promoter (which surrounds the SstI site; see above) and transcription proceeds through the inserts, ending at or near the normal ermC termination site. The tyc-1 transcript was indistinguishable in size from that of the wild type plasmid as expected (not shown). These results strongly support our previously reported conclusion that ermC regulation is posttranscriptional (8).

The tyc-1 and tyc-16 mutations have been further characterized by DNA sequencing. The tyc-1 plasmid has a single base change, converting position 317 from a C to an A. tyc-16 contains an extra 109 bp inserted between positions 321-322. The inserted DNA is a direct tandem duplication of the 109 residues immediately downstream from the point of insertion.

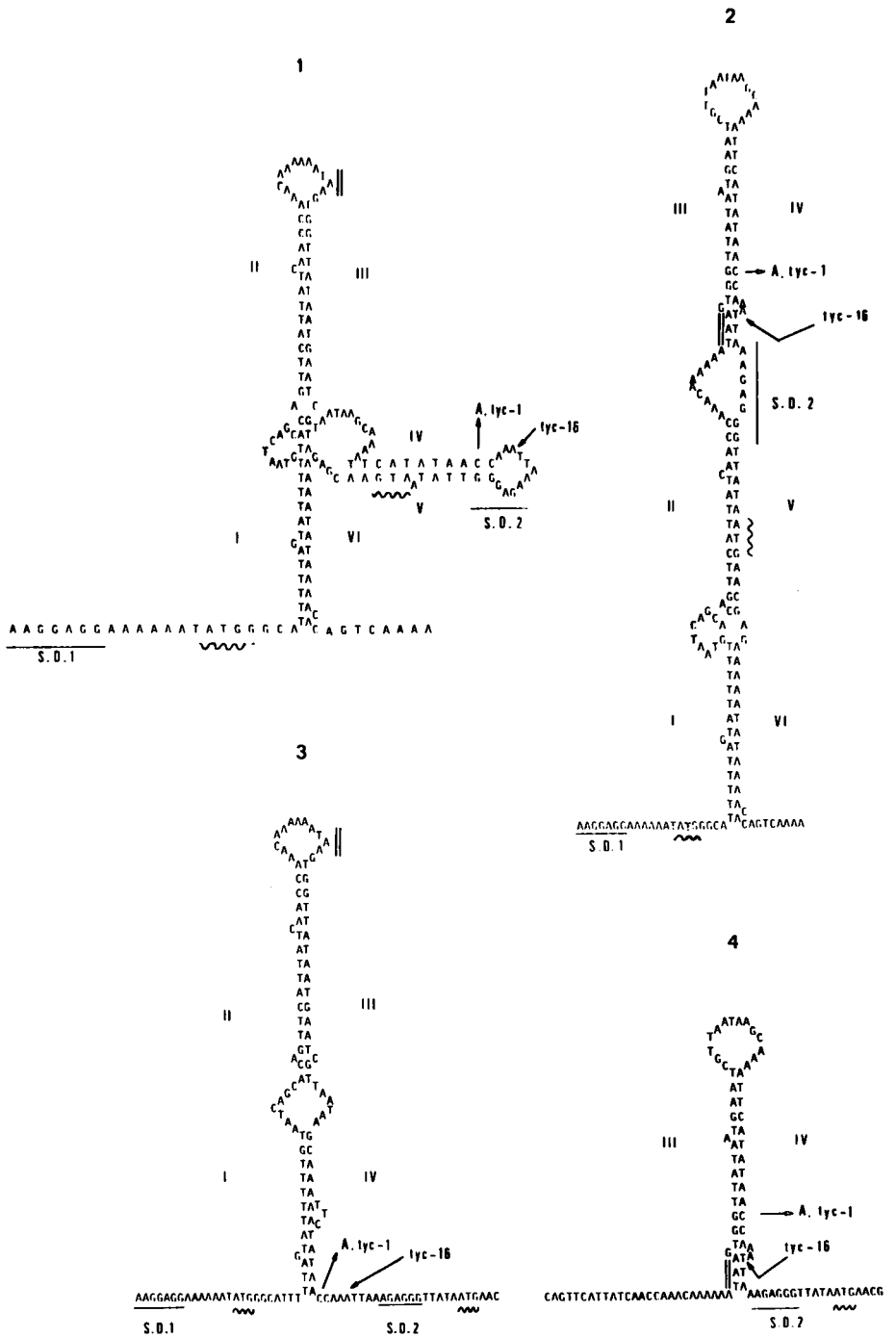
DISCUSSION

We have reported that Em induction of the 29K protein is mediated post-transcriptionally and that interaction of Em with its ribosomal binding site is necessary for induction to occur (8). Perturbation of this site by mutational alteration of protein L17 or by methylation of 23S rRNA, prevents induction.

Based on these observations, we postulated that the specificity of 29K protein regulation resides in changes in the structure of its mRNA (8). The present work serves to support and extend this hypothesis. We propose that the 141 base leader sequence, within which the tyc mutations occur, defines a cis-acting regulatory sequence for 29K protein translation. In accordance with this concept, we have demonstrated that several tyc mutations are dominant over the wild type allele (not shown).

Examination of the leader sequence and of the region just downstream from it reveals the presence of six repeat sequences, one of which (V) includes the ATG codon and part of the SD2 sequence. The repeats permit us to propose several hairpin structures for the 5' end of ermC mRNA (Fig. 5).

Based on these features we will present three models of induction by Em. All three rest on the notion that alternate conformational states of the mRNA control the initiation of 29K protein translation depending on whether the SD2 and ATG sequences are masked. Analogous models have been invoked to explain translational control in other systems (26). Structures 1 and 2


1
II
III
IV
V
VI
I
A. tyc-1
tyc-16
S.D. 2
S.D.1
2
III
IV
A. tyc-1
tyc-16
S.D. 2
II
V
I
VI
S.D. 1
3
II
III
I
IV
A. tyc-1
tyc-16
S.D.1
S.D.2
4
III
IV
A. tyc-1
tyc-16
S.D. 2

(Fig. 5) would thus be relatively inactive, whereas 3 and 4 would be active. Structure 1 appears more attractive than 2 as a candidate for the inactive form, since the tyc-1 mutation would destabilize the IV-V stem-loop by removing a GC·GC base stacking interaction, thus tending to unmask the SD2 and ATG sequences. In structure 2, the III-IV stem-loop would be weakened instead, without directly destabilizing the relevant portion (II-V) of this structure. Due to the tandem duplication, the tyc-16 mutation should permit the formation of structures similar to 1 and 2, but in which the SD2 and associated ATG codons are exposed (not shown). The tyc-16 mutation would thus be expected to increase the uninduced synthesis of 29K protein, as observed. The two suggested active structures would also be differently affected by the tyc-1 mutation. The III-IV stem of structure 4 should be destabilized, thus possibly mitigating the effect of destabilization of inactive structure 1. Structure 3, on the other hand, would be unaffected by the tyc-1 mutation.

It now remains to consider how Em may intervene to effect a conformational isomerization from an inactive to an active form.

Model I. Translational attenuation

Structures 1 and 2 both contain SD1 and its associated ATG codon in an exposed configuration. Ribosomes may thus initiate translation of the 19 amino acid peptide, terminating at the TAA signal which is preceded by two lys codons. This may result in transient destabilization of the inactive structure(s) and may facilitate isomerization to an active form, explaining the observed basal synthesis of 29K protein (6). In the presence of a subsaturating (inducing) concentration of Em (10-100 nM in the culture medium), movement of some of the ribosomes translating the 19 amino acid peptide may be slowed or blocked, causing them to accumulate within segments I and II, with a resulting shift to an active mRNA conformation. Since the majority of ribosomes will be Em-free at low concentrations of inducer, many of the ribosomes which can bind to the newly exposed SD2 sequence will not be inhibited. In vitro binding experiments have shown that B. subtilis ribosomes are half-saturated (0.5 mol Em bound/mol ribosomes) at an Em concentration of about

FIG. 5. Hypothetical hairpin structures for the 5' end of ermC mRNA. The sequences are written with thymine residues to facilitate comparison with Fig. 2. The locations of the tyc-1 base change and tyc-16 insertion are indicated. Other designations are as in Fig. 2. Structures 1 and 2 are proposed to be inactive, and 3 and 4 to be active, in 29K protein synthesis.

1 μM (8). Thus, when a low inducing concentration is used (and perhaps also transiently with high extracellular concentrations) this model is feasible, and presents a solution to the dilemma posed by induction of a new protein by Em, an inhibitor of protein synthesis. It should also be noted that the basal synthesis of 29K protein presumably results in the presence of a small proportion of methylated ribosomes, making possible a slow but exponential "escape" from Em inhibition of 29K protein synthesis, even at high intracellular concentrations. We have observed that strains carrying pE194 can form colonies on 8 mM Em. Induction by such elevated concentrations of antibiotic may occur by this "escape" mechanism. Thus, the basal synthesis of 29K protein may play an important biological role.

Several relevant points can be made concerning the mode of action of Em on protein synthesis (reviewed in [31]). This antibiotic does not inhibit formation of initiation complex. It can bind to polyribosomes only when the nascent peptide chain is short. Thus, once translation is under way, continued synthesis of a given peptide chain rapidly becomes Em resistant. This effect may also facilitate "escape" of 29K protein synthesis from inhibition as the intracellular Em concentration increases. On the other hand, once bound, Em and the related macrolide oleandomycin (Om) only inhibit transpeptidization when the nascent peptide chain exceeds a minimal size (31). Other macrolides, e.g., Ty and carbomycin (Cn), can block transpeptidization when the peptide chain is small. Em and Om induce 29K protein synthesis, whereas Ty and Cn do not (unpublished). This may be reasonably explained in terms of Model I, since the ribosomes must presumably traverse a minimum length before isomerization of mRNA to an active structure can occur. Ty and Cn may block translation before this minimum length is reached. It has been noted in model systems that the effect of Em on peptide bond formation is substrate-dependent. With many simple substrates no inhibition has been noted. Whereas Em actually stimulated the puromycin reaction with acetyl-Phe tRNA, polylysyl-puromycin synthesis was inhibited, leading to accumulation of di- and trilysylpeptides (32). The two lys codons situated just before the UAA stop signal for the 19 amino acid peptide may thus be a site of high sensitivity to Em, causing accumulation of ribosomes and leading to isomerization of the mRNA. These two codons may thus be analogous to the multiple amino acid codons observed in transcriptional attenuation systems (33-38).

Model II. Em-induced isomerization

Em may interact directly with an ermC mRNA-ribosome complex. The latter

complex may form weakly with an inactive mRNA structure (structures 1 and 2, Fig. 5), since only a portion of SD2 is exposed. Em may then perturb the environment of its binding site sufficiently to increase the unmasking of SD2. Isomerization of the mRNA to an active configuration may then be facilitated by the extensive homology of SD2 with the 3' terminus of 16S rRNA. This model is short on molecular specifics, although it is consistent with what we know about the ermC system.

Model III. Autoregulation

We have reported that synthesis of the ermC gene product is autoregulated and that mutational alteration of the 29K protein results in a hyperinducible phenotype (8). This autoregulation may be mediated by increased methylation of the 23S rRNA following induction of the 29K protein, thus blocking the required Em binding site. Alternatively, it may be due to direct feedback inhibition of translation by 29K protein. The latter effect could be mediated by interaction of the 29K protein with the mRNA-ribosome complex, resulting in stabilization of an inactive configuration, much as the R17 coat protein turns off replicase synthesis (reviewed in [26]). This inactive mRNA-ribosome-29K protein complex may form, as suggested in model II, using the partially exposed SD2 sequence, or even at SD1. Em may then enter its ribosomal binding site and interact with 29K protein, lifting the repression and facilitating mRNA isomerization to an active form.

We have recently reported that Em specifically stabilizes the ermC mRNA, increasing its functional half life at least tenfold (8). This may be an indirect effect; increased translation may secondarily result in protection of the ermC transcript. In any event, the stabilization effect will also serve to increase the rate of synthesis of the 29K protein upon induction.

ACKNOWLEDGMENTS

We gratefully acknowledge stimulating discussions with A.G. Shivakumar, Y. Kozlov, I. Smith, E. Dubnau, L. Mindich, R.P. Novick, and R. Losick. We thank A. Howard for expert and speedy secretarial assistance.

This work was supported by NIH grant AI-10311 and American Cancer Society grant VC-300, both awarded to D.D. G.G. was partially supported by funds from Farmitalia C. Erba S.p.A., Milan, Italy.

REFERENCES

1. Lai, C.-J., Dahlberg, J.E. and Weisblum, B. (1973) Biochemistry 12, 457-460

2. Lai, C.-J. and Weisblum, B. (1971) Proc. Natl. Acad. Sci. USA 68, 856-860
3. Lai, C.-J., Weisblum, B., Fahnestock, S.R. and Nomura, M. (1973) J. Mol. Biol. 74, 67-72
4. Iordanescu, S. (1976) Arch. Roum. Path. Exp. Microbiol. 35, 111-118
5. Weisblum, B., Graham, M.Y., Gryczan, T. and Dubnau, D. (1979). J. Bacteriol. 137, 635-643
6. Shivakumar, A.G., Hahn, J. and Dubnau, D. (1979) Plasmid 2, 279-289
7. Shivakumar, A.G., Gryczan, T.J., Kozlov, Y.I. and Dubnau, D. (1980) Molec. Gen. Genet. 179, 241-252
8. Shivakumar, A.G., Hahn, J., Grandi, G., Kozlov, Y. and Dubnau, D. (1980) Proc. Natl. Acad. Sci. USA 77, 3903-3907
9. Gryczan, T.J., Contente, S. and Dubnau, D. (1978) J. Bacteriol. 134, 318-329
10. Maxam, A.M. and Gilbert, W. (1980) in Methods in Enzymology, Grossman,L. and Moldave, K., Eds., Vol. 65, Part I, pp. 499-560, Academic Press, New York
11. Sanger, F. and Coulson, A.R. (1978) FEBS Lett. 87, 107-110
12. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251
13. Maniatis, T., Jeffrey, A. and Kleid, D. G. (1975) Proc. Natl. Acad. Sci. USA 72, 1184-1188
14. Maxwell, I.H., Maxwell, F. and Hahn, W.E. (1977) Nucleic Acids Res. 4, 241-246
15. Carmichael, G.G. and McMaster, G.K. (1980) in Methods in Enzymology, Grossman, L. and Moldave, K., Eds., Vol. 65, Part I, pp. 380-391, Academic Press, New York
16. Alwine, J.C., Kemp, D.J., Parker, B.A., Reiser, J., Renart, J., Stark, G.R. and Wahl, G.M. (1979) in Methods in Enzymology, Wu, R., Ed., Vol. 68, pp. 220-242. Academic Press, New York
17. Berk, A.J. and Sharp, P.A. (1977) Cell 12, 721-732
18. Favaloro, J., Treisman, R. and Kamen, R. (1980) in Methods in Enzymology, Grossman, L. and Moldave, K., Eds., Vol. 65, Part I, pp. 718-749, Academic Press, New York
19. Maniatis, T. and Efstratiadis, A. (1980) in Methods in Enzymology, Grossman, L. and Moldave, K., Eds., Vol 65, Part I, pp. 299-305, Academic Press, New York
20. Gryczan, T., Shivakumar, A.G. and Dubnau, D. (1980) J. Bacteriol. 141, 246-253
21. Rosenberg, M. and Court, D. (1979) Ann. Rev. Genet. 13, 319-353
22. Shine, J. and Dalgarno, L. (1974) Proc. Natl. Acad. Sci. USA 71,1342-1346
23. Shine, J. and Dalgarno, L. (1975) Nature 254, 34-38
24. Woese, C., Sogin, M., Stahl, D., Lewis, B.J. and Bowen, L. (1976) J. Mol. Evol. 7, 197-213
25. Sprague, K.U., Steitz, J.A., Grenley, R.M. and Stocking, C.E. (1977) Nature 267, 462-465
26. Steitz, J.A. (1979) in Biological Regulation and Development, Goldberger, R.F., Ed., Vol. 1, pp. 349-399, Plenum Press, New York
27. Talkington, C. and Pero, J. (1979) Proc. Natl. Acad. Sci. USA 76, 5465-5469
28. Haldenwang, W.G. and Losick, R. (1979) Nature 282, 256-260
29. Sutcliffe, J.G. (1978) Nucleic Acids Res. 5, 2721-2728
30. Contente, S. and Dubnau, D. (1979) Plasmid 2, 555-571
31. Pestka, S. (1977) in Molecular Mechanisms of Protein Biosynthesis, Weissbach, H. and Pestka, S., Eds., pp. 467-553, Academic Press, New York
32. Cerna, J., Rychlik, I. and Pulkrabek, P. (1969) Eur. J. Biochem. 9, 27-35
33. Barnes, W.M. (1978) Proc. Natl. Acad. Sci. USA 75, 4281-4285

34. DiNocera, P.P., Blasi, F., DiLauro, R., Frunzio, R., and Bruni, C.B. (1978) Proc. Natl. Acad. Sci. USA 75, 4276-4280
35. Zurawski, G., Brown, K., Killingly, D. and Yanofsky, C. (1978) Proc. Natl. Acad. Sci. USA 75, 4271-4275
36. Zurawski, G., Elseviers, D., Stauffer, G.V. and Yanofsky, C. (1978) Proc. Natl. Acad. Sci. USA 75, 5988-5992
37. Gardner, J.F. (1979) Proc. Natl. Acad. Sci. USA 76, 1706-1710
38. Gemmill, R.M., Wessler, S.R., Keller, E.B. and Calvo, J.M. (1979) Proc. Natl. Acad. Sci. USA 76, 4941-4945