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Ribosome Regulation by the Nascent Peptide

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INTRODUCTION

Control of mRNA translation has been established as the primary form of regulation for many bacterial and eukaryotic genes; often, translational control is complementary to transcriptional control (46, 58, 62). More than eight discrete mechanisms that allow modulation of the translation of particular species of mRNA are presently known (46, 62). These studies show that while the ribosome and mRNA are the central players in translation, the ribosome is typically not a target for regulation. One could speculate why this might be so. Ribosomes translate many different mRNAs; consequently, regulation of ribosome activity by *trans*-acting effector molecules could influence the expression of a wide range of genes. However, successful forms of gene regulation typically involve the modulation of "expression cassettes," which probably excludes *trans*-acting effectors of ribosomes as a general regulatory device. In contrast, selective regulation of the translation of individual genes can be achieved by binding specific molecules, protein or RNA, to target mRNAs (62).

Several independent observations obtained in recent years argue that the ribosome may be the target for a form of translational control in which the nascent peptide is the effector molecule (36, 37, 81, 94, 95). In this type of gene control, the nascent peptide affects only the ribosome that is involved in its

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own synthesis. Nascent peptide control of ribosome function is suggested by studies with bacterial and eukaryotic systems, although few have been extensively characterized at both the genetic and biochemical levels. Therefore, although the systems appear similar, underlying details may differ. In this review, we arbitrarily group the known examples of nascent peptide control into two categories depending on whether the regulatory peptide is specified by an independent coding sequence or is within the amino acid sequence encoded by the regulated gene.

cis-ACTING PEPTIDES SPECIFIED BY CODING SEQUENCES UPSTREAM FROM THE REGULATED GENE

Translation Attenuation of *cat* and *cmlA*

The most extensively studied system demonstrating *cis* effects of a nascent peptide on ribosome function involves translation attenuation regulation of chloramphenicol resistance genes in bacteria (80, 81). Translation attenuation controls the inducible expression of several antibiotic resistance genes (23, 24, 80, 137). Regulation appears to control translation exclusively (27). Genes regulated by translation attenuation are associated with a unique mRNA architecture, which provides a hint about the basis of this unusual control mechanism (Fig. 1). In each example, the ribosome-binding site (designated RBS-C) for the drug resistance coding sequence is within one member of a pair of inverted repeat sequences. Transcripts are therefore predicted to sequester RBS-C in a stable stem-loop structure in which it is unlikely to be available for translation initiation. Immediately upstream from the stem-loop structure is the leader coding sequence, which specifies a short peptide. Evidence from two translation attenuation systems, *cat* and *erm*, has shown that the stem-loop structure blocks translation of the resistance gene by preventing ribosome loading and that ribosome stalling in leader RNA destabilizes the stem-loop structure, thus freeing RBS-C for translation initiation (1, 25, 26, 50, 63, 80, 87). Induction requires the presence of subinhibitory levels of a specific, ribosomally targeted antibiotic, which is chloramphenicol for *cat* genes and erythromycin for *erm* genes. Induction can be remarkably rapid for the *catA86* gene, even when total RNA synthesis is blocked; near-maximum induction occurs within 15 min of addition of chloramphenicol to rifampin-blocked cells (27). It has long been suspected that the role of the antibiotic in induction is to stall a ribosome on the leader transcript (24, 80, 137).

Genes now known to be regulated by translation attenuation specify proteins that confer resistance to the antibiotic that is the inducer: *cat* genes specify chloramphenicol acetyltransferase (80, 117); *cmlA* encodes an apparent membrane protein that probably alters chloramphenicol transport (5, 9, 32, 114); and *erm* genes encode an enzyme that methylates a nucleotide in 23S rRNA, which prevents erythromycin from binding to the ribosome (68). However, the coding sequence for the drug resistance protein is dispensable for inducible regulation, as demonstrated by the antibiotic inducibility of *lacZ* translational fusions at early sites within the *erm*, *cat*, and *cmlA* structural genes (23, 24, 92, 137, 143). Therefore, sequences that allow a specific antibiotic to induce gene expression are entirely within the regulatory region that precedes the drug resistance coding sequence.

Requirement for site-specific ribosome stalling. Using the *catA86* operon as a model for translation attenuation (Fig. 1) (27, 29, 54), we showed that a ribosome must stall at a specific codon in the leader region of the transcripts to induce *cat* expression (1, 26). This was first demonstrated by starving *cat*-containing cells for the individual amino acids specified by the codons of the leader (1, 26). Amino acid starvation that forces a ribosome to stall with its aminoacyl (A) site at leader codon 6 activates translation of the downstream *cat* coding sequence (Fig. 2). Amino acid starvation that causes stalling with the A site at codons 3′ to leader codon 6 leads to a diminished induction response, and stalling of ribosomes at codons upstream from leader codon 6 does not induce (Fig. 2).

A potential problem in interpreting the above experiments is that translation of the *catA86* coding sequence requires all 20 amino acids (53). Therefore, when the cell is deprived of a leader-encoded amino acid, it is also deprived of an amino acid essential for translation of the reporter gene, *catA86*. A source of the deprived amino acid which is necessary for *catA86* translation seemed likely to be endogenous protein turnover that is induced by amino acid starvation (131). Amino acid starvation-induced host protein turnover is not observed in *rel* bacterial mutants (11, 119, 127, 131). Induction of *catA86* by amino acid starvation was therefore tested in a *rel* mutant of *Bacillus subtilis* (126). In the *rel* mutant, *catA86* could not be induced by lysine starvation (by using a *cat* mutant in which a lysine codon was present once in the leader as codon 6) unless lysine was supplied exogenously at a very low level (2). We suggest that the presence of trace amounts of lysine apparently allowed ribosome stalling at leader codon 6 in some of the *cat* transcripts while providing sufficient lysine levels for translation of *cat* in a portion of the induced transcripts (2). As would be expected, high lysine levels added to the *rel* mutant prevented *cat* activation, presumably by preventing ribosome stalling at leader codon 6 (2).

The correlation between ribosome stalling at leader codon 6 and induction of *cat* translation is also supported by the results of a different type of experiment. The earliest position of a nonsense codon in the *cat* leader that permits induction by chloramphenicol should define the location of the A site of an inducing ribosome. Replacement of *catA86* leader codon 2, 3, 4, or 5 with an ochre codon prevents induction by chloramphenicol (1), but ochre replacement of leader codon 6 permits chloramphenicol induction (Fig. 2) (1). Comparable results have been reported for the *catA112* gene (7). Thus, both amino acid starvation experiments and the ochre location experiment demonstrate that an inducing ribosome must stall with its A site at leader codon 6 for the induction of *cat* gene expression (Fig. 1).

The collective observations support a model for *cat* regulation by chloramphenicol (80) in which the antibiotic causes the translating ribosome to stall at leader codon 6, which destabilizes the adjacent stem-loop (52) and allows a second ribosome to initiate translation of the *cat* coding sequence (Fig. 3). Genetic analyses of the spatial relationship between a ribosome stalled at leader codon 6 and a ribosome initiating translation of *catA86* at RBS-C are consistent with this model (44). Shortening the intervening distance by deleting as few as 3 nucleotides (nt) from the loop diminishes basal and induced *cat* expression, suggesting partial interference of the stalled ribosome with the initiating ribosome (44). Deletion of 6 nt from the loop further reduces basal and induced expression, and deletion of 9 nt from the loop abolishes *cat* expression (44). Accordingly, in the wild-type *catA86* operon, the number of nucleotides separating leader codon 6 and RBS-C ensures that a ribosome stalled at leader codon 6 will not block a ribosome initiating at RBS-C.

It was also observed that increasing the distance between leader codon 6 and RBS-C by inserting up to 13 nt into the loop had no effect on induced expression but increased basal

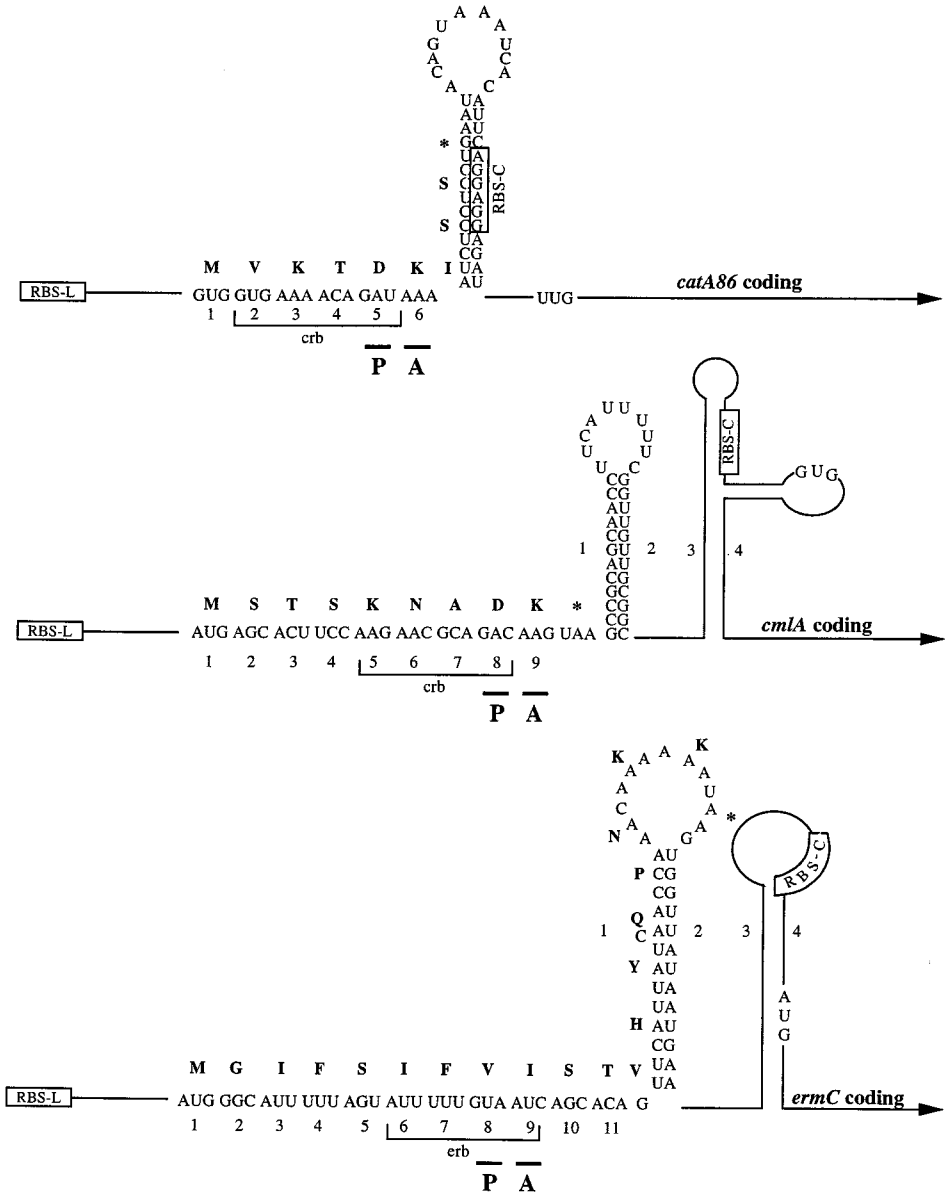

FIG. 1. Sequences toward the 5′ ends of *catA86*, *cmlA*, and *ermC* transcripts that confer regulation by translation attenuation. The domain of secondary structure sequesters the RBS for the drug resistance gene, blocking translation initiation. Ribosome stalling in the upstream-translated leader destabilizes the secondary structure, allowing translation of the resistance gene, either *cat*, *cmlA*, or *ermC*. Regulatory sequences for related inducible *cat* or *erm* genes are similar to that depicted for *catA86* and *ermC* (7, 24, 54, 59, 80, 118, 125, 137). RBS-L and RBS-C refer to the ribosome-binding sites for the leader and coding sequences, respectively. P and A refer to the peptidyl and aminoacyl sites, respectively, of a ribosome stalled in the leader by an inducing antibiotic (1, 26, 42, 63). *crb* encodes the portion of the *cat* and *cmlA* leader peptides that pauses ribosome elongation. *crb* is complementary to a region of *B. subtilis* 16S rRNA (107). *erb* is a region of the *ermC* leader that is thought to function is an analogous manner to *crb*. *erb* is weakly complementary to a region of *B. subtilis* 16S rRNA that overlaps the complement to *crb* (107).

(uninduced) expression up to fivefold (45). These results are consistent with the idea that basal expression of *catA86* is due to a ribosome translating the leader. As the A site of a ribosome passes through leader codon 6, the adjacent stem structure is destabilized, allowing the entry of a second ribosome at RBS-C. However, as the A site of the ribosome continues through leader codons 7 to 9, the leader ribosome sterically blocks ribosomes attempting initiation at RBS-C (Fig. 4). Accordingly, we believe that basal expression of wild-type *cat* is almost entirely the result of initiations at RBS-C which occur as the leader ribosome passes over leader codon 6 (Fig. 4). Inserting 3 nt into the loop allows a ribosome passing over leader codons 6 and 7 to destabilize the stem and not compete with entry of a ribosome at RBS-C. Hence, *cat* basal expression increases. A 6- or 9-nt loop insertion further enhances basal expression by allowing ribosome entry at RBS-C while the leader ribosome passes over leader codons 6 to 8 and 6 to 9, respectively. This interpretation of the results of Gu and Lovett (44) and additional genetic studies (108) are consistent with the two-ribosome model for translation attenuation regulation (Fig. 3) and are inconsistent with models that rely on a single ribosome for both destabilization of the stem-loop and initiation of *cat* translation (108).

Defining a ribosomal stall sequence, *crb*. While chloram-

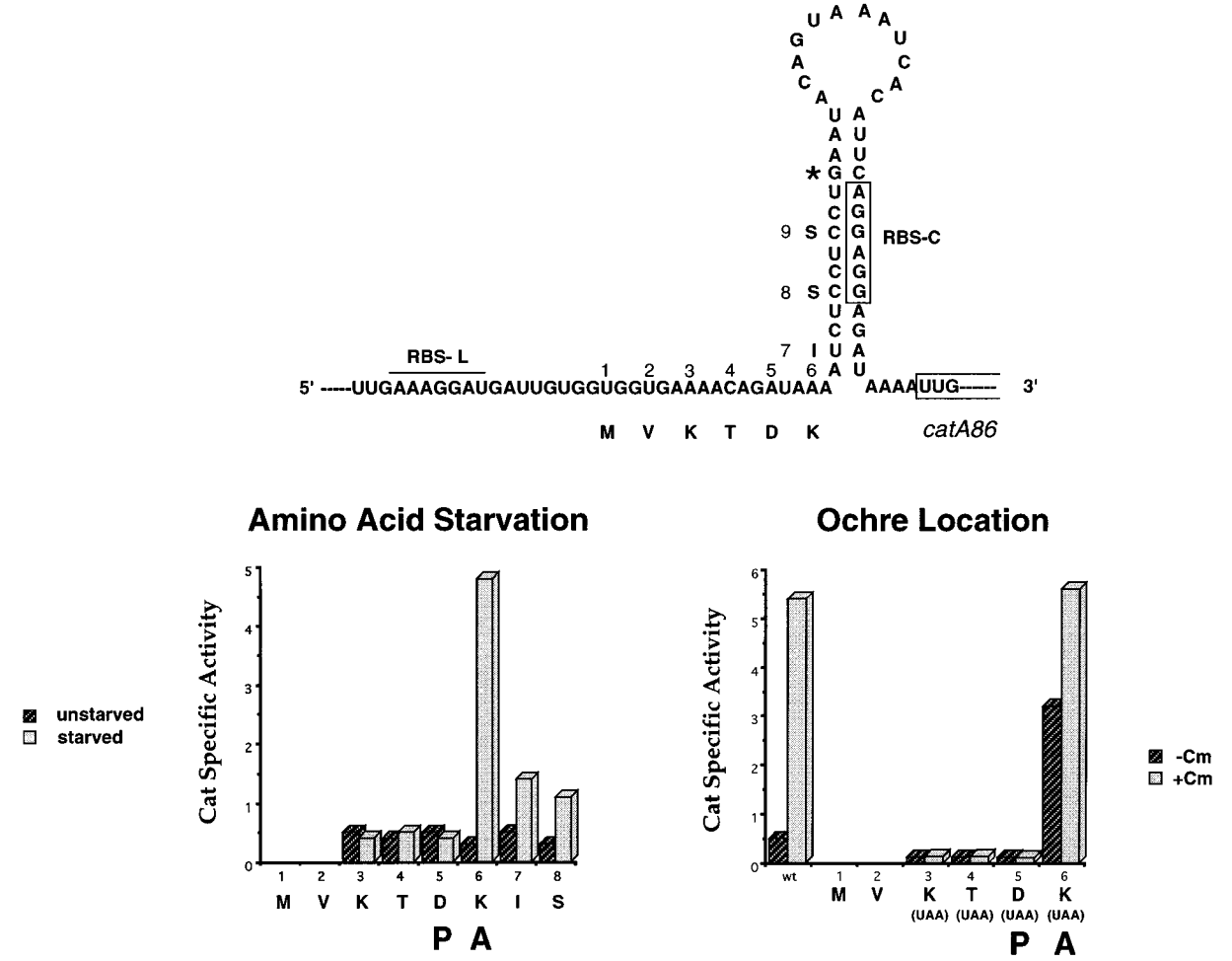

FIG. 2. The aminoacyl site of a ribosome must stall at leader codon 6 to induce translation of *catA86*. For amino acid starvation, a set of nearly isogenic mutants of *B. subtilis* was constructed, each mutant lacking the ability to synthesize one of the amino acids specified by the *catA86* leader. *catA86* was transformed into the mutant bacteria, and each strain was deprived of its required amino acid for 2 h at 37°C, at which time chloramphenicol acetyltransferase (Cat) was measured. Since Lys is represented twice in the leader, the effect of ribosome stalling at the first Lys codon, by starvation for lysine, was determined in *catA86* containing a mutation that switched leader codon 6 from a Lys codon to a Tyr codon. Stalling at the second Lys codon, by starvation for lysine, was assayed in a mutant in which the first Lys codon was switched to a Thr codon (1, 26). Symbols: ▨, unstarved; ⊡, starved. For the ochre location experiment, *catA86* leader codons 2 to 6 were individually mutated to the ochre codon. Each mutant was transformed into the same strain of *B. subtilis* and tested for induction by chloramphenicol (1). Symbols: ▨, minus chloramphenicol; ⊡, plus chloramphenicol.

phenicol and erythromycin are inhibitors of translation, neither is known to halt a ribosome at a particular nucleotide sequence in mRNA or at a specific amino acid sequence in a nascent protein. Therefore, two possibilities exist for the mechanism of action of the antibiotics as inducers. In the first, the inducing antibiotic might stall a ribosome at random sites within the leader. Induction would result from fortuitous stalling of a fraction of the ribosomes at the correct stall site, which is leader codon 6 for *cat* genes. The second explanation requires that a specific sequence exist upstream from *cat* leader codon 6 which selects the site of ribosome stalling. To distinguish between these models, a second copy of leader codon 5 (GAU) was inserted between leader codons 5 and 6 of the *catA86* operon (65, 109). This insertion abolished induction, which was restored by a subsequent deletion of leader codon 6 (AAA). Thus, nucleotides upstream from *cat* leader codon 6 appeared to select the site of ribosome stalling. These studies also show that the nature of leader codon 6 is probably not critical for induction, since chloramphenicol inducibility is retained when codon 6 is changed from the wild-type AAA (Lys) to either GAU (Asp) (65) or UAU (Tyr) (26).

To identify the codons of the stall sequence, which is designated *crb* (for chloramphenicol ribosome binding), a series of sequential codon insertions was made between leader codons 5 and 6 (Fig. 5). Chloramphenicol inducibility lost owing to insertion of leader codon 5 returned when the full inserted sequence consisted of codons 2 through 5 (109). Accordingly, leader codons 2 through 5 constitute the stall sequence *crb*. These experiments also showed that a *catA86* operon containing two *crb* sequences, one adjacent to the stem-loop and one further upstream, is induced by chloramphenicol but to only half the level seen with the wild-type *catA86* operon. Deletion of portions of the further-upstream *crb* sequence restored full inducibility, arguing for competition between the upstream and downstream *crb* sequences in chloramphenicol-dependent ribosome stalling (109). Therefore, *crb* appears functional as a stall site selector even when it is displaced from the stem-loop structure.

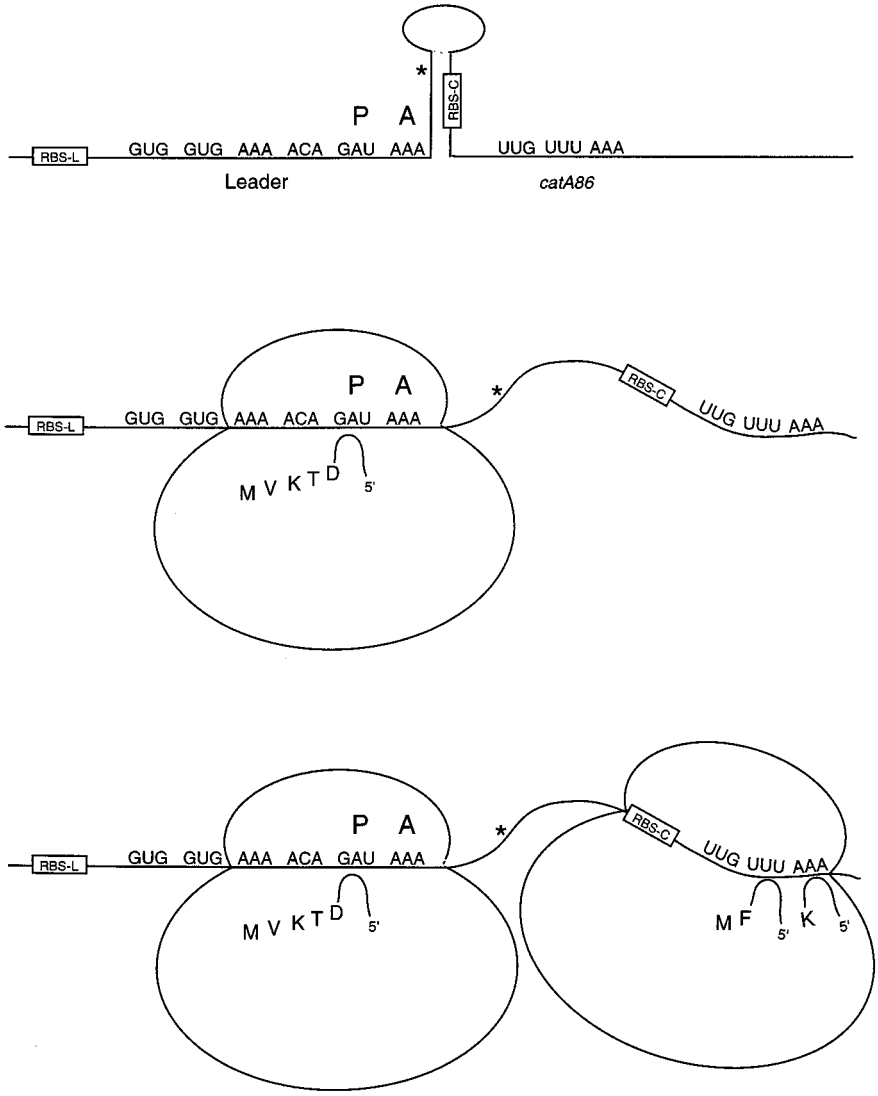

FIG. 3. Model for the induction of *catA86*. It is proposed that a ribosome stalled at leader codon 6 destabilizes the stem-loop structure, which frees RBS-C, allowing translation initiation of the *catA86* coding sequence.

***crb* function is not due to its nucleotide sequence.** Complementarity between *crb* nucleotides and rRNA was initially considered to be one mechanism that might enable a ribosome to recognize the stall site in leader mRNA (107). Conceivably, if such complementarity existed, it might trigger ribosome pausing. A search of *B. subtilis* rRNA for sequences complementary to *crb* (leader codons 2 to 5 of *catA86*) revealed a region of 16S rRNA complementary to 83% of the *crb* nucleotides (ΔG = −14 kcal/mol [−58.6 kJ/mol]) (107). The correlation between the complementarity of rRNA sequences with the leaders of genes regulated by translation attenuation extends to inducible *erm* and inducible *tet* genes; the leaders for both genes contain sequences complementary to a 30-nt region of 16S rRNA that contains the *crb* complement (107). Indeed, the regions in 16S rRNA that are complementary to *cat*, *erm*, and *tet* leaders overlap. Thus, it was (and remains) surprising that the complementarity of the *catA86* leader with 16S rRNA is not at all critical for the regulation. Cumulative third-position changes in the *crb* codons (synonymous codon changes) virtually abolished the proposed pairing (ΔG = −2 kcal/mol [−8.4 kJ/mol]) but reduced chloramphenicol inducibility by 50% or less (107). Although complementarity between 16S rRNA and the nucleotides of *crb* cannot be the primary mechanism through which the site of stalling is selected, the complementary sequences might facilitate stall site selection or stabilize a stalled ribosome on the mRNA.

The peptide product of *crb* is the stall factor. Several lines of evidence arguing that the leader-encoded peptide is the stall site selector have emerged. Specific missense mutations in the coding regions of leaders for regulated *cat* genes abolish induction (22, 43, 65, 111). As one example, a single nucleotide change in leader codon 4 changes the wild-type Thr codon (ACA) to a Pro codon (CCA) and abolishes chloramphenicol inducibility. Significantly, this mutation does not prevent induction brought about by amino acid starvation that stalls a ribosome's aminoacyl site at leader codon 6. Thus, the missense mutation disables drug induction but not induction brought about by artificially stalling a ribosome at the proper leader site.

A second class of mutations that eliminate chloramphenicol

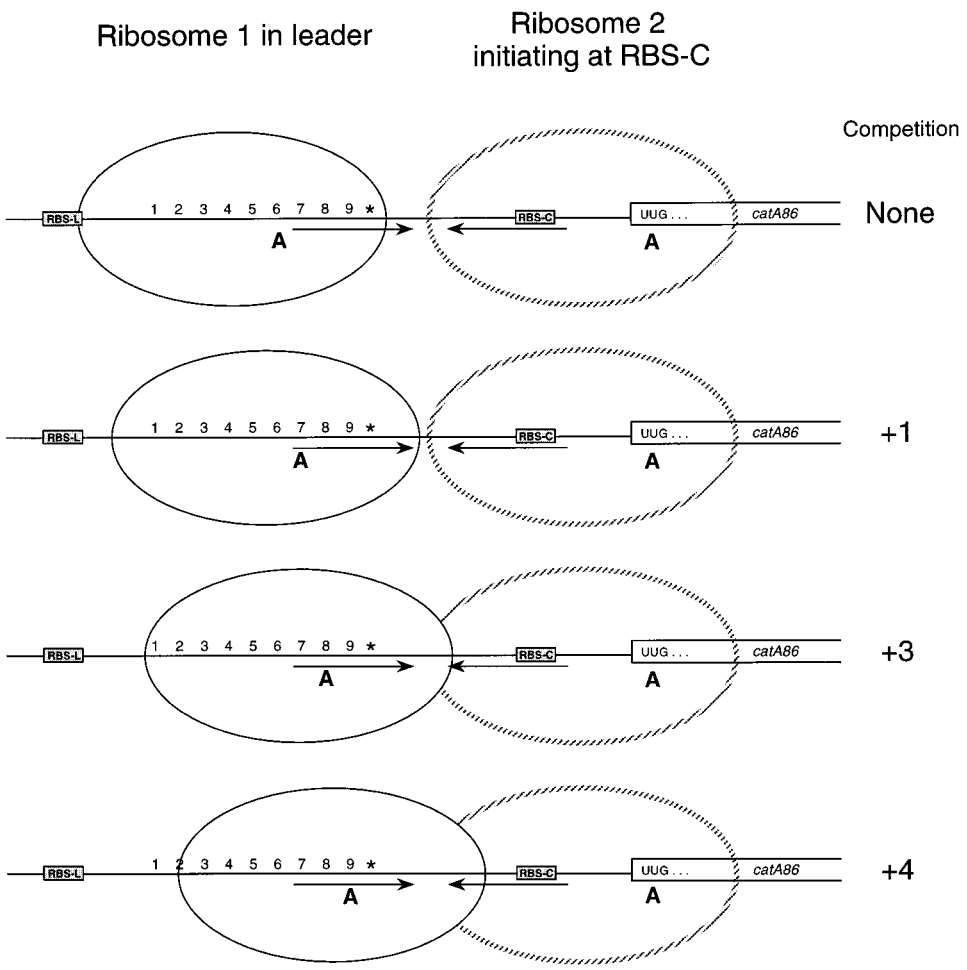

FIG. 4. Proposed effect of stalling the aminoacyl site of a ribosome at different leader codons on translation initiation at RBS-C. From the experiments of Alexieva et al. (1) and Gu and Lovett (44), we propose that a ribosome stalled with its A site at leader codon 6 can destabilize the stem-loop structure and not compete with entry of a ribosome at RBS-C. When the leader ribosome A site occupies leader codon 7, 8, or 9, the leader ribosome progressively encroaches on the space needed for a second ribosome to initiate at RBS-C. Consequently, a ribosome stalled at leader codons 3′ to leader codon 6 will impede or prevent the entry of a ribosome at RBS-C. This model explains why ribosome stalling at leader codon 6 is the ideal site for ribosome stalling that results in induction of *cat* translation. The model further predicts that basal (uninduced) *cat* expression is largely the result of initiation at RBS-C which occur as the leader ribosome translates through leader codon 6.

inducibility involves a change in the *crb* reading frame (107, 109). When the frame change is made 5′ to *crb*, induction is abolished, but when the frame change is 3′ to *crb*, inducibility by chloramphenicol is retained (107, 109). Therefore, it is the sequence of *crb* codons that is essential for inducibility by chloramphenicol. It is noteworthy that the addition of one, two, or three codons between leader codons 5 and 6 or simply the deletion of leader codon 6 prevents induction by chloramphenicol, arguing that inducibility requires maintenance of a precise spatial relationship between *crb* and the left inverted repeat. However, a single nucleotide insertion or deletion between leader codons 5 and 6 does not measurably alter induction. This finding indicates that the spacing between *crb* and the left leg of the stem cay vary by 1 nt and still support induction but the induction mechanism cannot tolerate a 3-nt change at the same location.

The third type of mutation in the leader that prevents induction is stop codon replacement of any of leader codons 2 to 5 (1). An ochre mutation at leader codon 3 (normally a Lys codon, AAA) abolishes chloramphenicol inducibility, but transformation of this mutant gene into a *B. subtilis* strain carrying the nonsense suppressor mutation *sup-3* restores chloramphenicol inducibility, albeit at a low level (96). The same suppressor mutation fails to restore inducibility when the ochre mutation is at leader codon 4 (normally a Thr codon, ACA) (96). Since *sup-3* inserts the amino acid lysine at ochre codons (96), it appears that the amino acid sequence of the leader encoded peptide is essential for induction by chloramphenicol.

Attempts to complement the loss of *cat* inducibility resulting from leader mutations by providing a copy of the wild-type leader in *trans* were unsuccessful (80). From this negative finding and recent studies cited below, we conclude that the leader-encoded peptide acts in *cis* on its translating ribosome.

Role for the *crb* peptide in *cat* autoinduction. Replacement of leader codon 6 of the *catA86* or *catA112* operons with the ochre codon (TAA) elevates basal (uninduced) expression five- to eightfold (1, 22) (Fig. 2); an identical result is obtained with the *catA86* operon when the stop codons are UAG or UGA (110). This phenomenon is termed autoinduction (110). Autoinduction is abolished when a stop codon mutation at leader codon 6 is combined with missense mutations in the leader that also prevent induction by chloramphenicol (110). This observation could be explained if the *cat* leader peptide

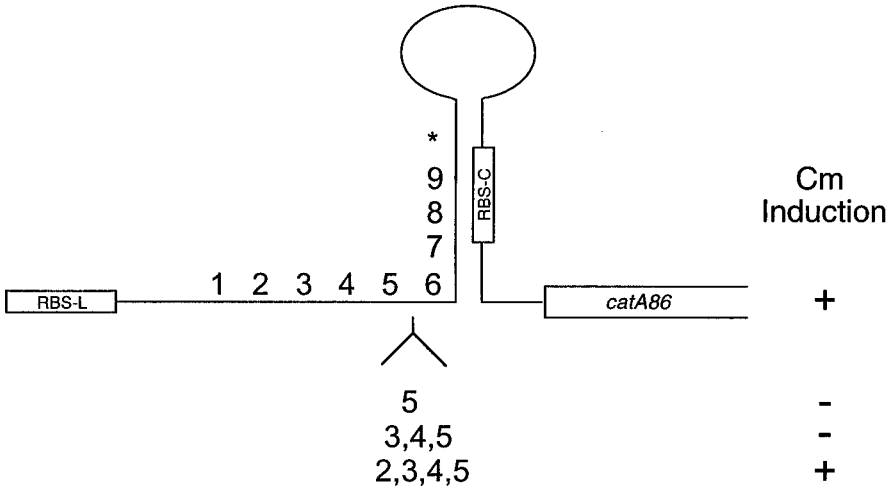

FIG. 5. Diagram of the effects of codon insertion mutations which define the *crb* stall sequence in the leader of *catA86* (109). Cm, chloramphenicol.

interfered with translation termination at the stop codon at leader codon 6 (110). The elevated basal expression of *cat* presumably results from long-term occupancy of the induction site in the absence of chloramphenicol. We suspect that the stop codon mutation at leader codon 6 forces a ribosome to pause at the induction site and that release of the ribosome from this location is prevented by interaction of the nascent, leader-encoded peptide with the ribosome (110). This interpretation predicts that the nascent leader peptide should be an inhibitor of ribosome release from the mRNA at the stop codon; that is, the leader peptide would inhibit translation termination (110).

In vitro peptide effects on ribosomes. A plausible mechanism exists through which the peptide product of the first five leader codons of *cat* operons might cause chloramphenicol to immediately stall the translating ribosome at the induction site. The nascent 5-mer peptide could interact, in *cis*, with its translating ribosome and enhance the affinity of the ribosome for chloramphenicol at the normal chloramphenicol-binding site on the ribosome. Alternatively, the peptide-ribosome interaction might provoke the appearance of a new, high-affinity binding site for chloramphenicol. Indeed, it has been shown that chloramphenicol induction of *cat* expression is not reversed upon removal of the antibiotic (106), indicating that induction by the drug differs from its action as a reversible inhibitor of protein synthesis. Although the above suggestions could explain the role of the leader peptide in induction, these models cannot account for its role in autoinduction. A direct effect of MVKTD on ribosome function was therefore predicted.

Peptidyltransferase is inhibited by MVKTD. Peptidyltransferase is the ribosomal catalytic activity that forms peptide bonds during translation (93, 98, 121). Antibiotic inhibition of this activity blocks protein synthesis by pausing or stalling translating ribosomes. Since a predicted function of the *cat* leader peptide, MVKTD, is to pause translation at the leader induction site, the ability of MVKTD to inhibit peptidyltransferase was examined. Peptidyltransferase activity resides within the peptidyltransferase center of the large ribosomal subunit and is assayed by the fragment reaction developed by Monro and Marcker (93), which monitors the transfer of *N*-formylmethionine from a T_1 nuclease-generated hexanucleotide fragment of initiator tRNA to puromycin. The reaction is catalyzed by an intact ribosome, the large ribosomal subunit, or specific fractions derived from the large subunit (see, e.g., reference 98).

When synthetic MVKTD peptide was added to ribosomes (47), peptidyltransferase activity was inhibited (Fig. 6). Inhibition was not observed with the reverse sequence, DTKVM. Therefore inhibition is not simply a function of peptide charge. C-terminal truncations of the pentapeptide such as MV, MVK, or MVKT were not inhibitors. Therefore, when a ribosome has translated to the leader induction site (the A site at leader codon 6), that ribosome has synthesized a 5-residue nascent peptide that is an inhibitor of peptide bond formation. Accordingly, we propose that the nascent peptide MVKTD selects the in vivo site of ribosome stalling by *cis* inhibition of peptidyltransferase. Consistent with this interpretation is the finding

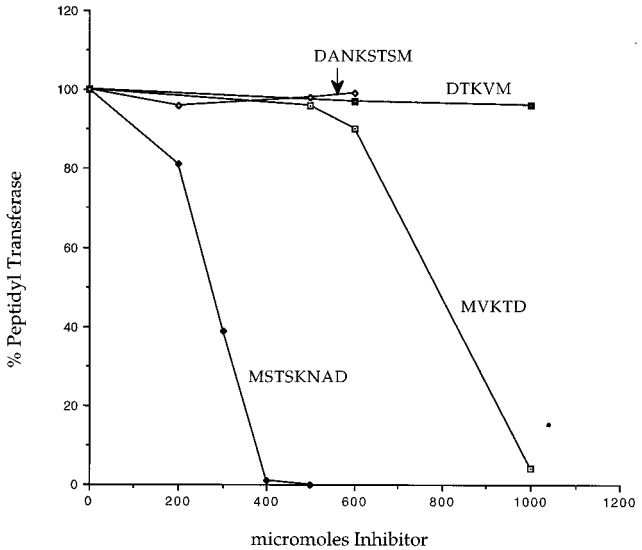

FIG. 6. Inhibition of peptidyltransferase by the peptides MVKTD and MSTSKNAD. Assays were performed as described by Gu et al. (43, 47), which is a slight modification of the Monro and Marcker procedure (93). The ribosomes used in this experiment were 80S ribosomes from *S. cerevisiae*. Comparable results have been obtained with ribosomes from either *B. subtilis* or *E. coli* (42, 47).

that missense mutations in the *catA86* leader that are known to diminish or abolish chloramphenicol induction in vivo correspond to amino acid sequence changes in the leader pentapeptide that also diminish or abolish inhibition of peptidyltransferase in vitro (43, 47).

Addition to ribosomes of increasing levels of the leader peptides demonstrates that a very high threshold level of peptide must be present before any peptidyltransferase inhibition is detected (Fig. 6). This finding may indicate that the peptides have low affinity for their target(s). Indeed, the concentration of MVKTD that is required for complete inhibition of peptidyltransferase, 1 to 2 mM, is high (47) (Fig. 6). On a molar basis, the inhibition of peptidyltransferase by the peptides is 20% or less of that by antibiotic inhibitors of peptidyltransferase such as lincomycin and amicetin (45). However, because the in vivo site of inhibitor peptide synthesis is also the site of action, the peptides may be more effective *cis* inhibitors of leader translation in vivo than the in vitro inhibition studies might indicate. The low specific inhibition observed when peptide is added to ribosomes in *trans* could be biologically significant, perhaps explaining why expression of the leader peptide in cells does not retard growth (107).

The peptides MKKAD and MKKSE specified by the leaders of other chloramphenicol-inducible *cat* genes (8, 59, 118) are also peptidyltransferase inhibitors and show a specific inhibition similar to that of the *catA86* peptide, MVKTD (42, 111). C-terminal truncations of these peptides, such as MKKA or MKKS, are not inhibitory for peptidyltransferase (42, 111).

Peptidyltransferase is inhibited by MSTSKNAD. The inducible chloramphenicol resistance gene *cmlA* identified in gram-negative bacteria encodes an apparent membrane protein and is thought to be regulated by translation attenuation (23, 125). The leader coding region of *cmlA* transcripts consists of nine codons and precedes a region of secondary structure that sequesters RBS-C of the *cmlA* coding sequence (Fig. 1). Codons 5 to 8 of the *cmlA* leader are similar to leader codons 2 to 5 of a composite *cat* leader 5-mer peptide sequence MKK(A/T/S)D (a consensus 5-mer peptide deduced from the leaders of *catA86*, *catA194*, *catA112/221* [8, 29, 59, 118]). Replacement of *catA86* leader codons 1 to 5 with *cmlA* leader codons 1 to 8 allows chloramphenicol induction of *catA86* in *B. subtilis* (42). The peptide MSTSKNAD, corresponding to the first eight codons of the *cmlA* leader, is an in vitro inhibitor of peptidyltransferase (Fig. 6) (42). The reverse sequence, DANKSTSM, is not inhibitory, nor is MSTSKNPD, in which a Pro replaces residue 7. Peptides corresponding to the first six or seven *cmlA* leader codons are also not inhibitory for peptidyltransferase (42). Thus, during the in vivo translation of the *cmlA* leader, an inhibitor peptide is generated only when a ribosome has translated to the induction site (the A site at leader codon 9). The *cmlA* 8-mer has a higher specific inhibition than the *cat* 5-mer does (Fig. 6). This difference seems to reflect a reduction in the threshold level of the 8-mer required for inhibition. Thus, perhaps the 8-mer is more inhibitory than the 5-mer as a result of differences in target affinity.

The ninth codon of the *cmlA* leader specifies Lys. Addition of Lys to the C terminus of the 8-mer, generating the 9-mer MSTSKNADK, eliminates peptidyltransferase inhibition (42). Therefore, the version of the *cmlA* leader peptide that is released from ribosomes after in vivo translation of the complete leader sequence is not a peptidyltransferase inhibitor. This could be an additional mechanism ensuring that overall host protein synthesis is unaffected by the leader peptide.

MVKTD inhibits translation termination. The phenomenon of autoinduction of *catA86*, which results from mutation of leader codon 6 to a translation termination codon, was suggested to result from inhibition of translation termination by the nascent leader peptide (110). This proposal was tested by determining if MVKTD could inhibit translation termination in vitro. MVKTD was inhibitory for translation termination, but DTKVM was not (91). Since MVKTD did not significantly inhibit the binding of release factor 1 to the ribosomes, the peptide must inhibit a subsequent step in termination such as release of the nascent peptide from P-site tRNA.

Inhibitor peptides interact with large-subunit rRNA. MVKTD and MSTSKNAD inhibit peptidyltransferase of ribosomes from eubacteria, archaea, and yeasts, indicating that the ribosomal target is likely to be a highly conserved sequence or structure (42). Phenol extraction of the rRNA from the large ribosomal subunit of either of two thermophilic bacteria, *Thermus aquaticus* and *B. stearothermophilus*, generates a form of 23S rRNA that is largely depleted of protein but is catalytically active in the peptidyltransferase assay (42, 98). This rRNA-enriched form of peptidyltransferase is inhibited by the 5- and 8-mer peptides (42, 43). Thus, it seemed likely that a target for the peptides would reside in the rRNA.

Certain ribosomally targeted antibiotics alter the relative reactivities of specific nucleotides in rRNA to methylation by dimethyl sulfate (DMS) (90). In a similar manner, the *cat* and *cmlA* leader peptides altered the response of specific nucleotides in *B. subtilis* large-subunit rRNA to DMS methylation (42). With the *Escherichia coli* numbering system for rRNA nucleotides, the 5- and 8-mer peptides prevented DMS methylation of nt 2058 and 2059 and enhanced DMS methylation of nt 2060. nt 2058 is also protected from DMS methylation by erythromycin (90), and erythromycin competed with the peptides for binding to rRNA (42). In vivo methylation of nt 2058 by the *erm* methylase confers erythromycin resistance. The peptidyltransferase activity of *erm*-methylated ribosomes retained susceptibility to inhibition by MVKTD and MSTSKNAD. The only obvious effect of *erm* methylation at nt 2058 was loss of erythromycin interference with peptide inhibition of peptidyltransferase. Therefore, nt 2058 seems unlikely to be a direct target for the peptides. Rather, peptide interaction at a different rRNA site may change the conformation of the rRNA, which indirectly prevents DMS from methylating nt 2058. Since erythromycin interferes with peptide effects on the rRNA but methylation at nt 2058 does not, bound erythromycin may be sufficiently bulky to prevent access of the peptide to its normal ribosomal target. Alternatively, erythromycin may prevent changes in ribosome or rRNA structure that are needed for peptide inhibition of peptidyltransferase.

Inhibitor peptides alter 23S rRNA conformation. To examine the interaction of the leader peptides with large-subunit rRNA directly, mixtures consisting of phenol-extracted *E. coli* rRNA and the peptides were probed with endoribonucleases that cleave at single-stranded (T_1) (Fig. 7) and double-stranded (V_1) RNA sites. The results demonstrated that MVKTD and MSTSKNAD altered the nuclease cleavage patterns only within domains IV and V of 23S rRNA (Fig. 8) (51). These are the rRNA domains at the peptidyltransferase center of ribosomes (6). Control peptides which do not inhibit peptidyltransferase did not alter the susceptibility of the rRNA to nuclease cleavage. Certain of the sites of altered nuclease susceptibility are at or adjacent to nucleotides to which a nascent peptide can be cross-linked during its exit from the ribosome (120). Therefore, sites of interaction of the inhibitor peptides with rRNA are consistent with their activity as peptidyltransferase inhibitors and with the path taken by a peptide as it passes through the ribosome during translation (6).

Do the inhibitor peptides interact with rRNA or with ribosomal proteins? Phenol extraction of rRNA appears to remove

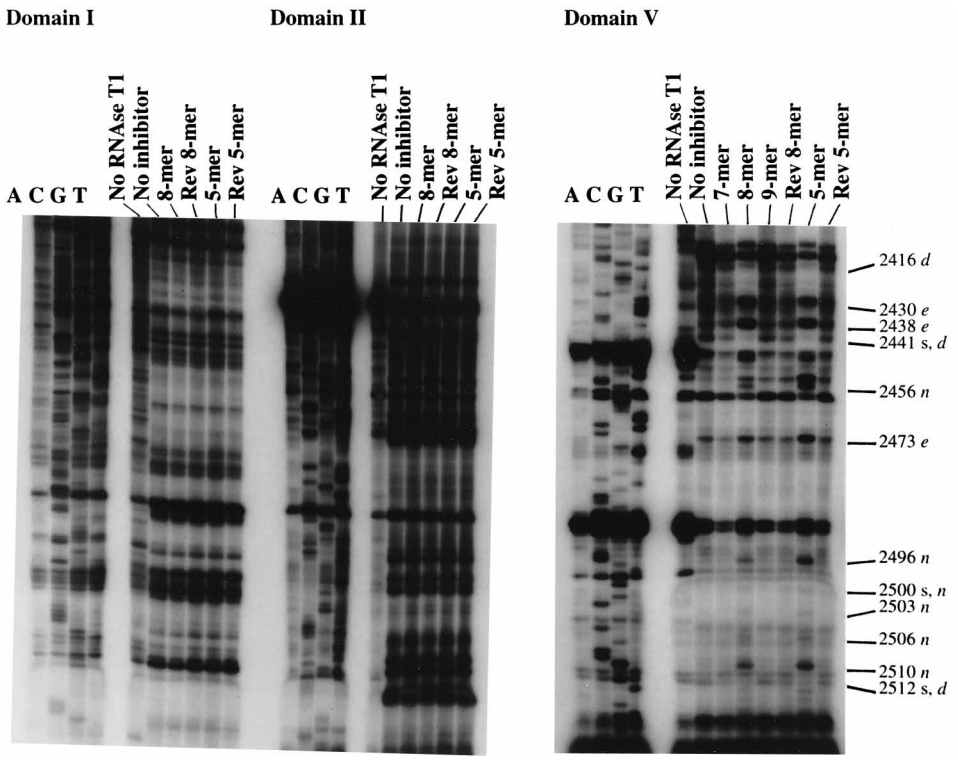

FIG. 7. Footprints of peptides on domain V of *E. coli* 23S rRNA. Phenol-extracted *E. coli* 23S rRNA was nicked with T1 endonuclease either alone or after incubation with the RNA with specific peptides. The sites of cleavage were detected by using reverse transcriptase and known DNA primers which are complementary for specific for regions of the RNA (51). The peptides alter the nuclease susceptibility within domains IV and V. Here we show three experiments, two of which are negative controls (domains I and II) and one of which demonstrates the types of peptide-induced changes observed (domain V). The numbers denote specific nucleotides, and the letters *d*, *e*, *s*, and *n* refer to diminished cleavage, enhanced cleavage, secondary-structure effect, and new site of cleavage, respectively. The 5-mer sequence is MVKTD, and the 8-mer sequence is MSTSKNAD. Both of these peptides alter the domain V secondary structure. Rev 5-mer is the reverse sequence of the 5-mer, DTKVM, which does not alter the rRNA secondary structure. The peptides labeled 7-mer and 9-mer are MSATSKNA and MSTSKNASK, respectively. Neither alters rRNA secondary structure. RNA-sequencing ladders shown in the figure were generated with reverse transcriptase. Therefore, the nucleotides seen in the ladders are the complements of the nucleotides in the rRNA. The right panel of this figure is from reference 51. The left two panels are negative controls reported, but not shown, in reference 51. Figure courtesy of Rob Harrod.

most but not all associated ribosomal protein (97, 98). To determine if residual ribosomal protein might be needed for peptide binding to the rRNA, the ability of the leader peptides to alter the conformation of domain V of in vitro-transcribed *E. coli* 23S rRNA was examined. MVKTD and MSTSKNAD caused a similar change in T_1 cleavage of domain V in both rRNA extracted from cells and rRNA transcribed in vitro (51). Thus, the binding of the leader peptides to the rRNA is independent of ribosomal proteins.

MVKTD and MSTSKNAD also alter the susceptibility of yeast and *Halobacterium* large-subunit rRNA to T_1 and V_1 nuclease cleavage in a manner comparable to that observed with *E. coli* 23S rRNA (52), suggesting that peptide inhibition of peptidyltransferase and translation termination is a consequence of peptide interference with rRNA structure.

Role of Leader Peptides and Antibiotics in Translation Attenuation

Translation by a ribosome to the induction site in the leaders of the *catA86* and *cmlA* operons occurs concomitantly with the synthesis of a nascent 5- or 8-residue peptide that is capable of binding to rRNA and inhibiting ribosomal peptidyltransferase (Fig. 9). It is thought that the inhibitory leader peptides pause translation in vivo immediately upon their synthesis (47). Since the resulting site of ribosome pausing is identical to the site at which ribosome stalling induces downstream translation, it is presumed that the inducer chloramphenicol converts a paused ribosome (a reversible state of cessation of translation elongation) to a stalled condition (nonreversible cessation of elongation), which locks a ribosome at the induction site (106).

Second function: antibiotic cooperation. Genes regulated by translation attenuation, such as *cat* and *erm*, are typically induced by very specific, ribosomally targeted antibiotics. The nature of the antibiotic that induces gene expression depends not on the drug resistance coding sequence but on the nature of the leader sequence (92, 116, 141). For example, *catA86* is induced by the ribosomal antibiotic amicetin as well as by chloramphenicol, although *catA86* confers resistance only to chloramphenicol (28). Of two classes of mutations which have been identified that prevent amicetin from inducing *catA86*, one is in the *catA86* leader. A mutation that changes leader codon 2 from GUG (Val) to AAA (Lys) prevents amicetin from acting as an inducer, but the mutation does not interfere with chloramphenicol inducibility (65). Furthermore, a synthetic peptide, MKKTD, having the amino acid sequence encoded by the amicetin-noninducible mutant is inhibitory for peptidyltransferase (43). Thus, the amino acid sequence of the leader peptide appears to determine whether amicetin can serve as inducer.

The second class of mutation that prevents *catA86* induction by amicetin is within a bacterial gene. This mutation, *ami-1*, affects the structure of the large-subunit ribosomal protein L12a and confers amicetin resistance on *B. subtilis* (28). The

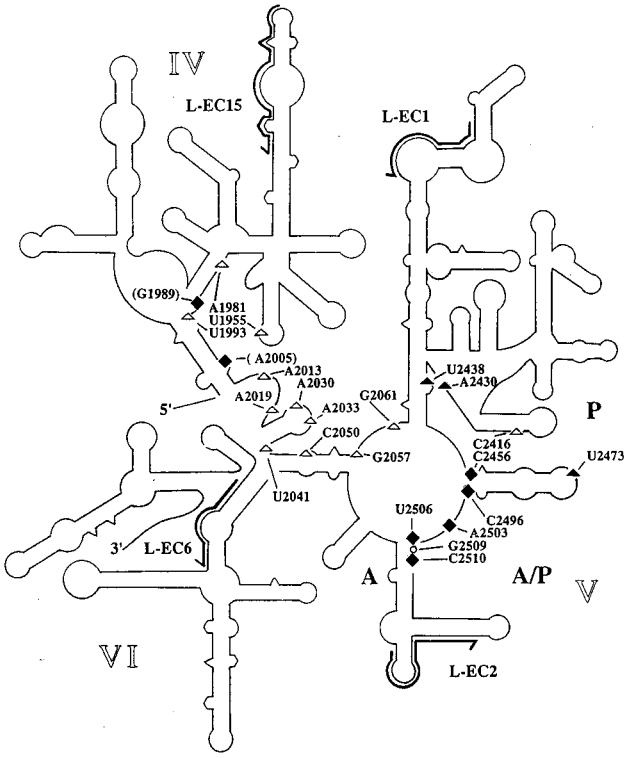

FIG. 8. Stick diagram of domains IV, V, and VI of *E. coli* 23S rRNA showing sites of peptide-caused alterations in response to T_1 or V_1 nuclease cleavage. Solid diamonds, new T_1 site; solid triangles, enhanced T_1 cleavage; open triangles, diminished or absent T_1 site; open circle, loss of V_1 site. The figure was generated from data in reference 51. The peptides MVKTD and MSTSKNAD altered the nuclease susceptibility of the rRNA identically.

ami-1 mutation does not diminish inducibility by chloramphenicol (28). *ami-1* has recently been shown to reduce the in vitro sensitivity of ribosomes to amicetin inhibition of peptidyltransferase (45).

The foregoing suggest that a leader peptide must satisfy two criteria to allow induction by a specific antibiotic. First, the peptide must be capable of binding in *cis* to its translating ribosome. The binding elicits a change in the ribosome, pausing translation. Second, the amino acid sequence of the leader-encoded peptide must cooperate with a ribosomal antibiotic if that antibiotic is to serve as the inducer. The nature of the proposed cooperation between the leader peptide and the inducing antibiotic is not known, although we suspect that the peptide may modify the response of the ribosome to the inducer. This suggestion therefore predicts that the combination of peptide and antibiotic has a different effect on ribosomes than does the antibiotic alone.

***erm*-encoded leader peptide.** Studies of *ermC* argue for a role of the encoded leader peptide in translation attenuation regulation of that gene. The leader must be translated for induction (25), and the leader-encoded amino acids seem critical for the regulation (86). Amino acid starvation that artificially stalls a ribosome at leader codon 9 will induce the gene (63), although starvation for the amino acids specified by flanking codons has not been tested. Unfortunately, the *ermC* leader peptide is insoluble in the buffer systems commonly used to study peptide interaction with ribosomes or rRNA (111). Therefore, studies of the *ermC*-encoded leader peptide will require a modification of the approach used to examine the in vitro activities of the *cat* and *cmlA* peptides.

In addition to *ermC*, several other inducible *erm* genes have been identified (75). Given the general similarity among the regulatory regions for these genes, it has been supposed that each follows the *ermC* model of translation attenuation. The finding that *ermK* is regulated by transcription attenuation is therefore unusual (75). The regulatory region of another *erm* gene, *ermD*, is strikingly similar to that of *ermK* and is inferred to also be regulated by a transcription attenuation mechanism (75). A leader peptide signal for ribosome stall site selection is compatible with regulation by either translation or transcription attenuation, although the latter imposes an additional requirement. In a transcription attenuation model, the mRNA secondary structure functions as a rho-independent transcription terminator and ribosome stalling in the leader alters the conformation of the terminator to an inactive form, allowing readthrough transcription (77). For ribosome stalling in the leader to have an effect on downstream transcription, the ribosome must stall prior to drop-off from the mRNA of the leading RNA polymerase molecule. Thus, the general model we propose for translation attenuation of *cat* and *cmlA* could apply equally well to stall site selection in a system regulated by transcription attenuation.

Other *cis*-Acting Peptide Effectors

The regulated bacterial operons *cat* and *cmlA* consist of two consecutive open reading frames (ORFs) on the same transcript (42); the leader ORF is short and is followed by a larger ORF specifying the antibiotic resistance protein. This pattern of gene organization is also seen in operons regulated by transcription attenuation in bacteria (77) and among proto-oncogenes, growth factor genes, and growth factor receptor genes commonly encountered in eukaryotes (69, 71). In eukaryotic genes that are preceded by a short ORF, the ORF is typically referred to as an upstream ORF (uORF) rather than as a leader. The translation of certain uORFs can inhibit translation of the downstream (major) ORF, and in specific examples, inhibition depends on the amino acid sequence of the uORF-encoded peptide (37). The latter clearly distinguishes this form of translational control from that governing translation of *GCN4* in *Saccharomyces cerevisiae* (58). In addition, among many studies of transcription attenuation in bacteria, one example has been identified in which the leader-encoded peptide seems to participate actively in the inducible transcription of the downstream coding sequence (40). The following examples illustrate the range of genes whose expression is altered by the peptide product of an upstream coding sequence.

***CPA1* gene of *Saccharomyces cerevisiae*.** One of the earliest examples of inhibition of translation as a result of a uORF in a eukaryote is seen in the yeast gene *CPA1*, encoding the small subunit of the arginine biosynthetic enzyme carbamoylphosphate synthetase (104). Synthesis of the protein is under two levels of regulation. First, the enzyme is subject to general amino acid control, which regulates at the transcriptional level (88, 89). Second, repression of *CPA1* expression by arginine is partially the result of translational control (16, 76, 89, 103). The nearly fivefold inhibition of *CPA1* expression by arginine is eliminated when translation of a 25-codon uORF is prevented by mutation of its initiation codon, by shortening the encoded peptide through the introduction of premature stop codons, or by missense mutations at uORF codon 11 or 13 (130, 139). By contrast, synonymous codon replacements within the uORF have virtually no effect on the inhibition, arguing that inhibition is probably a function of the uORF encoded peptide.

The insertion of the uORF at sites upstream of *GCN4* or *ARG4* causes expression of these coding sequences to be in-

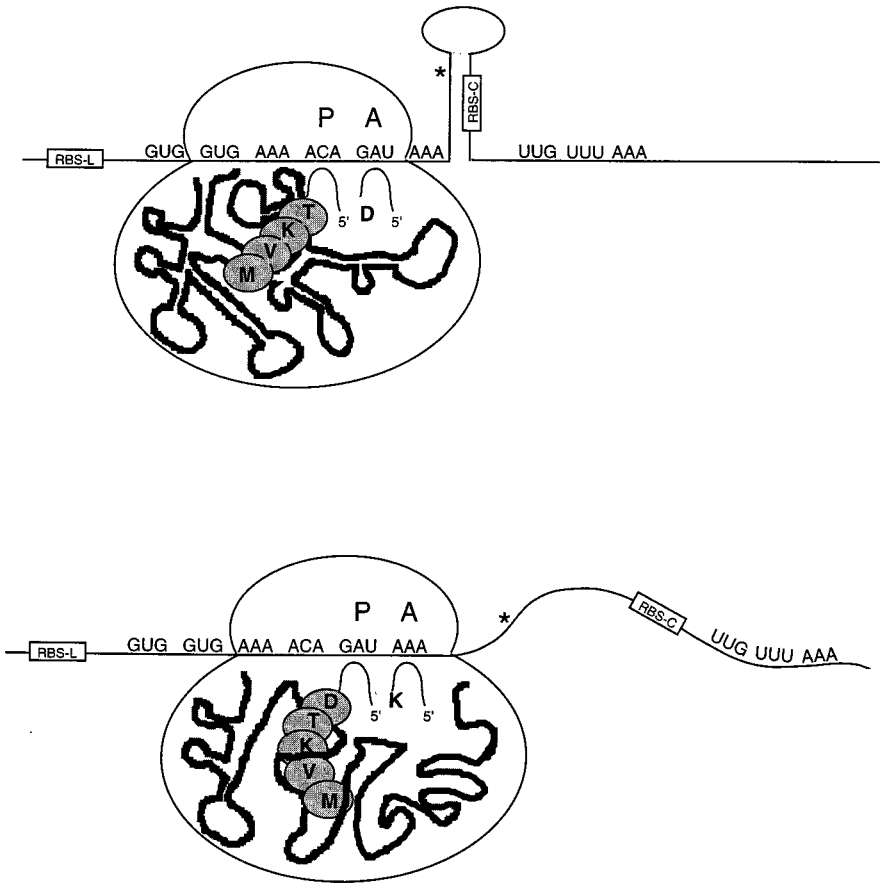

FIG. 9. Model for a pausing effect of the nascent leader peptide on its translating ribosome. When MVKTD is translated, the peptide interacts with 23S rRNA with a concomitant inhibitory effect on peptidyltransferase and translation termination (47). The asterisk represents the translation stop codon that terminates the leader coding sequence.

hibited by arginine (21). Additionally, fusion of the uORF and *lacZ* produces a hybrid gene that is also arginine inhibited. A missense mutation at uORF codon 11, known to relieve arginine inhibition of *CPA1* expression, also partially relieves arginine inhibition of the uORF-*lacZ* hybrid gene (21).

Missense mutations in *CPA1* which prevent arginine inhibition reside in the uORF and are *cis* dominant (139). Thus, interpretations of the role of the peptide in arginine inhibition of *CPA1* translation have focused on its influence on the ribosome that has just translated the uORF. To explain the *cis*-dominant behavior of the uORF, Delbecq et al. (21) suggested that the encoded peptide may be unstable and may function as a translation inhibitor only at its site of synthesis.

(i) Geballe-Morris proposal. A comprehensive model proposed by Geballe and Morris (37) may explain how the *CPA1* uORF peptide and other eukaryotic uORF peptides inhibit downstream translation. In this model, the suppressive activity of a uORF peptide depends on the mechanism required for most known translation initiation in eukaryotes. Translation initiation in eukaryotes typically follows the scanning model (70), which differs from the targeted mechanism through which translation initiates in bacteria. Scanning relies on the entry of a 43S preinitiation complex at the 5′ CAP structure of eukaryotic mRNAs. The complex migrates in the 3′ direction and initiates translation at the first AUG codon. Geballe and Morris (37) have suggested that the nascent uORF-encoded peptide acts on its translating ribosome and prevents release at the termination codon for the uORF. This stalled ribosome sets up a blockade to ribosome scanning, preventing initiation of translation of downstream coding sequences. The as yet untested prediction of this model is that a uORF-encoded peptide will interfere with termination of translation in vitro, either alone or in combination with the coeffector, which is arginine for *CPA1* (37). The proposed antitranslation termination activity would be equivalent to that demonstrated for the *catA86* leader peptide MVKTD (91, 110).

***arg-2* gene of *Neurospora crassa*.** *arg-2* of *Neurospora crassa* encodes the small subunit of carbamoyl-phosphate synthetase and therefore is functionally analogous to the *CPA1* gene of *S. cerevisiae* described above. Among the *N. crassa* arginine biosynthetic genes, only *arg-2* expression is negatively regulated by arginine (18). Repression by arginine diminishes *arg-2* protein and mRNA but short term exposure to arginine appears to effect a change only in translation (83). The *arg-2* coding sequence is preceded by a 24-codon uORF (100), and two classes of mutations in the uORF eliminate arginine-mediated inhibition of translation of the *arg-2* coding sequence. By use of a uORF-*lacZ* fusion, it was shown that removal of the uORF initiation codon prevented arginine inhibition (83). In a second series of experiments, a hygromycin fusion to the uORF was used to select for a mutation that relieved arginine inhibition in vivo (31). The *cis*-acting mutation (31) was shown to result from a missense mutation at codon 12 of the uORF, which changed an Asp codon to an Asn codon.

Comparison of the sequence of the *arg-2* uORF with uORFs of several eukaryotic genes and with the leaders of *catA86* and *cmlA* shows the presence of an Asp codon toward the 3′ end of the short coding sequence (31). When this codon was changed to one specifying a nonacidic amino acid, *cis* regulation was lost. However, an Asp codon is not present at a similar location in the *catA112/221* leader or, as shown below, in the uORFs of cytomegalovirus CMV gp48, the β_2-adrenergic receptor gene, and the *Lc* gene of maize (see Table 1). Therefore, it is unclear if the presence of a 3′ Asp codon (or even a Glu codon) might signal a type of regulation by the encoded peptide that differs from that by peptides lacking this amino acid.

Freitag et al. (31) interpret their findings of *cis* regulation of *arg-2* translation in light of the Geballe and Morris proposal (37). They suggest that the uORF-encoded peptide interacts with its translating ribosome to block ribosome release at the uORF stop codon, thus setting up a blockade to ribosome scanning to the *arg-2* coding sequence (31). Apparently, arginine must participate in the regulation, perhaps by functioning as a coeffector at the level of the ribosome or by a more complex sequence of events.

gp48 gene of cytomegalovirus. Cytomegalovirus (CMV) is a member of the herpesvirus family and causes severe infections of humans, especially of immunocompromised individuals. An understanding of the basic mechanisms regulating gene expression in CMV is therefore of great importance. Several CMV genes are preceded by short ORFs, and analysis of one gene, gp48, has identified a uORF that interferes with gp48 translation.

gp48 specifies a structural glycoprotein of the virion (12). Of three uORFs that precede gp48, deletion analysis identified that uORF2 alone was responsible for inhibition of the expression of a gp48-*lacZ* hybrid gene during transient assays of virus-sensitive cells. The level of mRNA was relatively constant among the deletion mutants generated, and primer extension analyses demonstrated that mRNA was being initiated at the appropriate start point for each mutant. It was therefore suspected that the uORF interfered with translation of the reporter gene. This interpretation was supported by the observation that transcripts of reporter gene constructs containing the wild-type uORF2 were markedly diminished on large polysomes compared with transcripts of reporter constructs that lacked the uORF2 inhibitory signal (12).

Deletions made immediately 3′ to the AUG of uORF2 relieved inhibition of reporter gene translation, as did mutations that changed the reading frame within uORF2 (115). Furthermore, missense mutations in the carboxy-terminal codons of uORF2 eliminated inhibition but synonymous codon changes did not (20). It was concluded that uORF2 down-regulation of translation of the gp48 coding sequence was a function of the uORF2-encoded 22-residue peptide. To test the ability of uORF2 to function in *trans*, cells were cotransfected with two different plasmid constructions, one lacking a functional uORF2 and having a short deletion at the 3′ end of the reporter gene and the other containing a functional uORF2 and having a wild-type reporter gene. The polysome sedimentation profile indicated that transcripts *cis* to the defective uORF2 sedimented with large polysomes whereas transcripts *cis* to the functional uORF2 sedimented with mono- and disomes (20). These data suggest that the uORF peptide functions only in *cis.*

Using the toeprinting technique, which detects the location of bound ribosomes (or proteins) on mRNA (53), Cao and Geballe (10) demonstrated that the uORF causes ribosome arrest on the mRNA at the location predicted by the in vivo studies. Missense mutations that relieve inhibition of translation in vivo also eliminate the toeprint in vitro, consistent with the proposal that the uORF peptide interacts with its translating ribosome to block its release at the uORF stop codon.

Two obvious questions remain unanswered in this and several subsequent studies of down-regulation by a uORF. First, is there a biological reason for down-regulating translation (of gp48 in this example)? Second, why was the proposed ribosome blockade mechanism chosen over alternative devices that should also reduce gp48 translation? Answers to these basic questions may reveal additional roles for regulatory uORFs in the modulation of gene expression.

***S*-Adenosylmethionine decarboxylase gene.** *S* -Adenosylmethionine decarboxylase (AdoMetDC) is essential for the synthesis of polyamines in eukaryotic cells, and the level of this enzyme is greatly reduced in T lymphocytes relative to other cell types (85). In T lymphocytes, AdoMetDC mRNA is associated primarily with monosomes, whereas the same mRNA in nonlymphoid cells carries seven to nine ribosomes per transcript (57, 85). A six-codon uORF is immediately 5′ to the AdoMetDC coding sequence. Mutations that decrease translation of the uORF and those that change the coding specificity of the last three uORF codons relieve inhibition of AdoMetDC translation in T lymphocytes (56). In contrast, six mutations in the uORF that result in synonymous changes of the codons do not diminish repression of AdoMetDC translation, suggesting the uORF effect is mediated through its peptide product (56). The uORF appears to be a *cis*-acting regulator, since complementation is not observed in *trans* in an assay system analogous to that used for dominance studies of the gp48 uORF. The authors propose a plausible model for suppression of AdoMetDC translation by the uORF in which the uORF-encoded peptide interferes with ribosome release at the uORF termination codon, setting up a ribosome blockade to scanning.

uORF-mediated inhibition of AdoMetDC translation is cell type specific. The lack of *cis* repression by the uORF in nonlymphoid cells occurs because uORF translation in this cell type is infrequent (113). Increasing the efficiency of uORF translation in nonlymphoid cells was accomplished by extending the distance between the uORF and the CAP structure by 47 nt; this resulted in uORF inhibition in vivo (113). Thus, the lack of uORF inhibition in nonlymphoid cells is due to the lack of synthesis of the uORF-encoded 6-residue peptide.

β_2-Adrenergic receptor gene. AUG codons occur infrequently in the 5′ leaders of vertebrate transcripts (71). However, the occurrence seems to be more frequent in transcripts that specify certain classes of proteins including members of the G-protein-coupled receptor superfamily (69). The β_2-adrenergic receptor is typical of this class of gene, and the leader region encodes a short ORF (sORF) (66). Inspection of sORFs preceding the β_2-adrenergic receptor genes of four vertebrate species (mouse, human, hamster, and rat) demonstrated a largely conserved nucleotide and codon sequence which specifies a highly basic 19-residue peptide (102). The murine sORF inhibits expression of the downstream receptor coding sequence. Mutation of the sORF AUG initiation codon to CUU eliminated inhibitory activity in vivo and in an in vitro translation system. The mutation did not diminish the level of receptor mRNA detected in vivo. Thus, it was concluded that the sORF inhibits downstream translation (66). Moreover, this experiment strongly suggested that inhibition was probably not solely the result of the nucleotide sequence of the sORF.

To further test the possible significance of the sORF-encoded peptide in the inhibition of receptor translation, three adjacent Arg codons of sORF were mutated to codons for Ala. These changes increased receptor translation by 50%. Furthermore, replacing sORF codon 4, CCA, with the translation stop

codon UGA enhanced receptor translation by 65%. Thus, all data suggest involvement of the sORF peptide in blocking downstream translation (66).

To determine if the sORF-encoded peptide would inhibit translation in *trans*, a β_2-adrenergic receptor bearing a nontranslatable sORF (CUU as the initiation codon) was cotransfected into COS-7 cells with the mouse α_2-10H-adrenergic receptor. The observed ratio of β_2-adrenergic receptor to α_2-adrenergic receptor was 1.8 times greater than when the wild-type β_2-adrenergic receptor gene was cotransfected with the α_2-adrenergic receptor gene. The authors suggest that the sORF-encoded peptide is not a *trans*-acting peptide; otherwise, sORF inactivation would have enhanced expression of β_2- and α_2-adrenergic receptors to the same extent (102).

The sORF-encoded peptide inhibits β_2-adrenergic receptor translation in vitro (102). A peptide concentration of 100 μM reduces translation by 99% (102). In contrast, a 10-fold-higher level of a mutant peptide bearing three Ala residues in place of the three Arg residues is needed to achieve a similar level of inhibition.

The authors (102) suggest the sORF-encoded peptide may impede receptor translation in vivo by interacting with its mRNA and setting up a block that diminishes ribosome scanning to the receptor initiation codon. The conclusions derived from the studies of the *cat* and *cmlA* leader peptides also suggest the translating ribosome or rRNA as a possible target.

***Lc* gene of maize.** *Lc* is a member of the *R/B* family of transcriptional activators, which are essential for the expression of pigmentation genes in maize. Different alleles of the *R/B* family cause different pigmentation patterns, and this is thought to reflect differences in the regulation of allelic expression rather than differences in the coding sequences. *Lc* is the first member of the *R/B* family to be sequenced (82). The *Lc* gene contains a uORF that consists of 38 codons. To study the role of the *Lc* uORF, mutations that prevent or modify uORF translation were introduced (17). To test the in vivo effect of the mutations, a plasmid containing the *Lc* gene with a uORF mutation was introduced into aleurone tissue along with a reporter plasmid that contained a luciferase gene fused to a promoter that requires the *Lc* gene product for transcription activation. In this system, fluorescence provides an indirect measure of *Lc* expression. Several modifications of the uORF were found to increase the expression of the reporter gene by 12- to 30-fold, compared with the level of fluorescence observed when a wild-type *Lc* gene was present with the reporter gene-containing plasmid. The mutations relieving the inhibition of *Lc* expression include deletion of the uORF, individual replacement of the three AUG codons of the uORF with Arg codons, and replacement of the first AUG codon with a stop codon. A nonconservative change of a Leu codon in the uORF to an Arg codon increased the expression of the reporter gene 23-fold, and conservative changes (Leu to Ile, Thr to Ser) and a synonymous codon change also elevated the expression of the reporter gene by 12- to 15-fold.

The uORF appears not to inhibit *Lc* translation in *trans*, since expression of the wild-type uORF sequence on one plasmid does not prevent expression of an *Lc* gene fully derepressed as a result of multiple uORF mutations present on a second plasmid (17). Comparison of the levels of *Lc* mRNA between the wild-type gene and two derepressed mutants showed no significant differences. Thus, the differences in expression are unlikely to be due to changes in transcription or mRNA stability.

It is conceivable that the repression of *Lc* translation might be due to uORF interference with translation termination at the uORF stop codon (37, 91, 110). However, since conservative and synonymous codon changes in the uORF also resulted in a 12- to 15-fold increase in *Lc* expression, it seems probable that the nucleotide sequence of the uORF also participates in the regulation. For instance, the nucleotide sequence might stabilize the ribosome on the mRNA through interactions with rRNA (e.g., see reference 107).

Tryptophanase operon of *E. coli*. Transcription attenuation controls the expression of a variety of bacterial genes, although it is perhaps best known as a regulator of transcription of amino acid biosynthetic operons (77). Transcription attenuation requires translation of a leader but does not appear to be dependent on the overall amino acid sequence of the encoded peptide. Rather, critical codons are positioned at sites within the leader such that a ribosome stalled at these codons will block the formation of a downstream transcription termination signal, the attenuator. This allows upstream-initiated transcription to enter downstream coding sequences. The nature of critical codons is related to the regulated operon. In the leader for the tryptophan operon, two adjacent tryptophan codons constitute the critical codons. A deficiency in tryptophan causes a ribosome to pause at the tryptophan codons, and that ribosome interferes with formation of the attenuator structure (77). That a leader-encoded peptide may actively participate in transcription attenuation regulation is therefore uncommon. Regulation of the *E. coli* tryptophanase operon provides the first such example.

Tryptophanase is a catabolic enzyme that degrades tryptophan. In *E. coli*, the gene for tryptophanase, *tnaA*, is within an operon that also contains the *tnaB* gene encoding a tryptophan permease (19). The structural genes of the operon are preceded by a 24-codon ORF, the leader, designated *tnaC*. Expression of the *tna* operon is induced 10- to 100-fold by tryptophan but not by other amino acids (123). Transcription attenuation appears to be the only form of regulation controlling the inducibility of *tna*.

Mutations in *rho* elevate *tna* operon expression (124), and deletion of a rho-utilization site immediately 3′ to the *tnaC* coding sequence causes constitutive expression of the *tna* operon (124). Therefore, termination in the leader is mediated by rho and antitermination requires tryptophan. The role of tryptophan as an antiterminator is unclear. Since induction (antitermination) is also observed with the tryptophan analogs 1-methyltryptophan and 5-methyltryptophan, which are very poorly charged onto tRNA, it is unlikely that a charged tryptophanyl-tRNA is directly involved in antitermination (41, 145).

tnaC is essential for tryptophan induction of the *tna* operon. Mutations that prevent translation of *tnaC* block induction, and the presence of a tryptophan residue as amino acid 12 of the leader encoded peptide is essential for induction (122, 124). Codons flanking the single *tnaC* tryptophan codon are identical in *E. coli* and *Proteus vulgaris* and similar in *Enterobacter aerogenes* (40). Synonymous codon changes in this conserved region of *tnaC* permit induction, but missense mutations that generate nonconservative amino acid replacements in the product peptide do not (40). Missense mutations in *tnaC* that prevent induction could not be complemented in *trans* by a wild-type copy of *tnaC* (39), suggesting that the *tnaC* encoded peptide may act in *cis*. However, the interpretation is complicated by the finding that *tnaC* at high copy number inhibits induction of the *tna* operon in the chromosome. Furthermore, *tnaC* at high copy number slows the growth of cells (40). Both effects could be accounted for if the *tnaC*-encoded peptide bound an essential cell component. Since ribosome pausing in leaders seems to be the general mechanism used in antitermi-

nation, it is conceivable that the *tnaC* peptide might have an affinity for a component of the ribosome.

Analyses of the data have led to the suggestion that the *tnaC*-encoded peptide may interact in *cis* with its translating ribosome. In conjunction with tryptophan, access of rho to targets in leader mRNA is blocked, presumably through ribosomal shielding (40). *cis* effects of the *tnaC* peptide on its translating ribosome therefore influence downstream transcription but not translation.

cis-ACTING PEPTIDES ENCODED BY DOMAINS WITHIN THE CODING SEQUENCE FOR A PROTEIN

Regulation of ribosome function by a leader or uORF-encoded peptide demonstrates a role for small nascent peptides in influencing translation in *cis*. It seems plausible that stretches of amino acids within proteins might also have *cis*-regulatory effects on translation. Ribosome pausing during the translation of long coding sequences has been reported (64, 105), although in the examples cited, the basis is not known to result from a *cis* effect of a nascent peptide. In contrast, the following cases demonstrate peptide sequences within proteins that affect ribosome activity in *cis.*

Topoisomerase Gene of Bacteriophage T4

Gene 60 encodes one of the three subunits of T4 topoisomerase (79). The gene 60 product is an 18-kDa protein. The coding region for gene 60 contains an internal sequence of 50 nt for which the product protein shows no corresponding amino acid sequence; the "coding gap" intervenes between codons 46 and 47 (61). Bypassing of the coding gap is observed during in vitro or in vivo translation of a gene 60-*lacZ* fusion. Splicing was clearly ruled out as a possible explanation for gap bypass.

Several features of gene 60 mRNA are required for gap bypass (ribosome hopping) in *E. coli* (138). First, a stop codon must follow the 5′ takeoff site for ribosome hopping. Second, the 5′ takeoff and 3′ landing codons which flank the nontranslated 50 nt must match. Third, the distance separating codons 46 and 47 must be about 50 nt. Fourth, a short hairpin structure in the mRNA must occur at the takeoff site, although a missense mutation in the gene for L9 ribosomal protein can substitute for the hairpin in hopping (55). Finally, it was shown that amino acids 17 to 32 of the topoisomerase protein, which are located well upstream from the takeoff codon, are critical for hopping. It has been speculated that during translation, the nascent peptide may bind to its translating ribosome with "functional consequences" (138). Analogy is drawn with the mode of action of the oligopeptide antibiotic edeine, which blocks P-site binding of tRNA (138). However, there are no in vitro tests of the activity of the nascent 16-residue peptide on ribosome function.

Since the coding-gap sequences are not critical for translation, it is not obvious why the gene has retained these sequences. In the absence of contradictory information, it must be presumed that the coding gap is somehow beneficial to T4 phage. For instance, bypassing of the coding gap forces the immediate upstream nascent peptide to retain a relatively fixed amino acid sequence and requires maintenance of other unique features of mRNA architecture essential for ribosome hopping (138).

Rhodanese

Rhodanese is a 33-kDa protein that is found in mitochondria (60). The protein is probably essential and functions as a sulfur transferase. Rhodanese is synthesized on cytoplasmic ribosomes and is subsequently transported to mitochrondria (49). In the process of transport and folding, the N-terminal Met is lost. The N-terminal 31 amino acids of rhodanese contain peptide sequences that target the protein to the mitochondria. Moreover, the structure of rhodanese, determined by X-ray crystallography, has demonstrated that the protein consists of two globular domains, with the N-terminal 22 residues positioned on top of one of these domains.

Kudlicki et al. (72) demonstrated that during the in vitro transcription-translation of rhodanese in a system derived from *E. coli* components, about half of the synthesized but enzymatically inactive protein remained associated with ribosomes. Release of enzymatically active protein from the ribosomes was observed on the addition of ATP and the chaperones GroEL, GroES, DnaK, DnaJ, GrpE; omission of ATP prevented release. GroEL, GroES, or DnaK added individually promoted the release of apparently full-length but catalytically inactive protein. Sparsomycin, a peptidyltransferase inhibitor, inhibited chaperone-mediated protein release and activation of catalytic activity; puromycin released the ribosome-bound protein, which remained inactive even after addition of chaperones and ATP. These data suggest that rhodanese remains attached to ribosomes as a peptidyl-tRNA–protein–ribosome complex in the absence of chaperones. It is inferred that the ribosome-associated form of the enzyme is incompletely or improperly folded, preventing detectable catalytic activity, and that this folding requires the participation of five *E. coli* chaperones. Since the addition of the chaperones releases the protein from ribosomes and simultaneously restores catalytic activity, proper protein folding may be essential both for catalysis and protein release. The identification of chaperones as the critical component for protein release from the ribosomes argues that the association of the nearly half of the synthesized protein with ribosomes may simply be due to the exhaustion of one or more of the chaperones during the early period of transcription-translation.

A version of the rhodanese gene deleted for the N-terminal 23 codons was more efficiently translated and released from ribosomes than was the wild-type protein (73). However, the deleted protein was catalytically inactive and improperly folded, and chaperone addition could not restore enzymatic activity. Moreover, a synthetic peptide corresponding to the N-terminal 17 amino acids of rhodanese inhibited the in vitro synthesis of the wild-type rhodanese but had little effect on synthesis of rhodanese deleted for the first 23 codons. Inhibition by the synthetic peptide appears to be due to inhibition of chaperone-mediated release of the completed rhodanese protein from ribosomes.

The collective data suggest that the N-terminal region of rhodanese is essential to proper folding of the nascent protein on the ribosomes and that this folding requires ATP and the participation of several chaperones; DnaK and GroES seem most critical (72). Proper folding of the nascent protein is essential for its release from ribosomes. The mechanism responsible for inhibition of release is suggested by the recent observation that the N-terminal 17-amino-acid peptide is an inhibitor of peptidyltransferase when DnaJ is also present (74). Thus, apparently if the N-terminal portion of rhodanese is not occupied as part of the correctly folded enzyme structure, its presence may antagonize the release of the nascent protein from ribosomes.

β-Tubulin

Microtubules participate in a wide variety of events within eukaryotic cells such as mitosis and motility. Microtubules consist primarily of the α and β subunits of tubulin, a heterodimer that exists in equilibrium with the microtubule polymer. Tubulin synthesis is coupled to tubulin polymerization into microtubules. When the intracellular levels of α- and β-tubulin are increased by microinjection of tubulin subunits or by depolymerizing microtubules with drugs, the synthesis of α- and β-tubulin ceases, and this correlates with a decrease in tubulin mRNA (3, 14, 15).

The regulation of synthesis of the tubulin subunits does not appear to be autoregulated at the level of transcription (129). Rather, several lines of evidence indicate that high levels of free tubulin subunits selectively alter the stability but not the synthesis of tubulin mRNA (34, 101). Fusion of as few as the first 13 nt of the β-tubulin coding sequence to thymidine kinase conferred on the hybrid mRNA tubulin subunit-induced instability (35, 146). However, instability is observed only on transcripts that are actively translated and requires that translation extend beyond codon 41.

Nucleotide changes in the first 4 codons of β-tubulin that result in synonymous codon changes did not alter the autoregulated instability, whereas nucleotide substitutions that changed the N-terminal 4 amino acids abolished regulation (147). It has been proposed that when the first 4 amino acids of β-tubulin emerge from the ribosome, they have the opportunity to contact a cellular component that is elevated when tubulin subunits are in excess. This interaction triggers a subsequent event that decreases the stability of tubulin mRNA which is under active translation (129). Initially, it was thought that direct contact between the first 4 amino acids of β-tubulin, MREI, and free tubulin subunits might be the initiating event. However, no interaction between the nascent peptide MREI and tubulin subunits could be demonstrated. More recent studies demonstrate that antibody to MREI does block tubulin subunit-induced mRNA instability, suggesting that an additional, as yet unidentified cellular component(s) is involved (129).

Signal Sequences and Signal Recognition Particles

One view of *cis* regulation by a nascent peptide is that the regulation is observed only when the peptide is within the target ribosome. The rhodanese and β-tubulin systems are therefore exceptions, since in both cases the nascent peptide exerts its regulatory effect only after the peptide has emerged from its translating ribosome. Signal sequences and signal recognition particles (SRP) similarly exert a *cis* effect on translation after the nascent peptide has emerged from its translating ribosome. The roles of SRP and signal peptides in the translocation of a translating ribosome to the membrane have been extensively reviewed (84, 140, 142) and will be mentioned here only briefly.

Proteins destined to be secreted from cells typically possess an N-terminal signal sequence consisting of 7 to 20 hydrophobic amino acids. When the signal sequence has emerged from its translating ribosome, it is bound by SRP, a ribonucleoprotein. The structure of SRP from various organisms has been extensively studied. The first characterized SRP is from mammalian cells and consists of six polypeptides and a 7S RNA (133–135).

After SRP has bound a signal sequence, the SRP-nascent peptide-translating ribosome complex is translocated to an SRP-docking site in the membrane, where the nascent peptide is released from SRP concomitant with insertion of the peptide into the membrane. The initial binding of SRP to the nascent peptide arrests translation, and release of SRP from the peptide at the docking site allows translation to resume. Accordingly, translation occurs simultaneously with translocation of the nascent protein through the membrane.

The signal sequence functions at two levels. First, it provides a recognition target allowing SRP to bind to a translation complex that is destined to produce a secreted or membrane protein. Second, the hydrophobic character of the amino acids of the signal sequence is critical to its insertion into a membrane.

HYPOTHESES FOR THE MODE OF ACTION OF NASCENT PEPTIDES AS *cis* REGULATORS

The activities demonstrated or proposed for the action of the regulatory peptides described above suggest two categories of nascent peptide control. In the first, the peptide has a direct effect on a function of the translating ribosome. In the second, the peptide plus another cellular component or environmental agent provokes a change of function in the translating ribosome. Our evidence suggests that translation attenuation regulation of *cat* and *cmlA* probably requires the leader peptide to function at both levels (42).

Peptides as Effectors

The *cat* and *cmlA* leader peptides (MVKTD and MSTSK NAD) are rRNA-binding peptides that inhibit peptidyltransferase and translation termination activities in vitro and support drug induction in vivo. Synthesis of the 5- and 8-mer peptides in vivo correlates with translation of the leader to a site where ribosome stalling will induce downstream translation. These findings argue for a direct *cis* effect of the *cat* and *cmlA* leader peptides on translating ribosomes as selectors of the site of ribosome stalling in the respective leader coding sequences. Several examples of uORF regulation of downstream translation in eukaryotic systems can also be explained by a direct effect of the nascent peptide on its translating ribosome (37).

Comparison of the sequences of the biologically active peptides that regulate translation (or transcription [38]) of unrelated genes shows no obvious similarities, with the exception that none is acidic (Table 1). It is therefore possible that the different peptides interact with the ribosome through different routes. Certain of the uORF-encoded peptides could bind to sites in rRNA different from those recognized by the *cat* and *cmlA* leader peptides (51), or the uORF peptides might bind to ribosomal proteins instead of or in addition to rRNA.

Since the regulatory target for the *cat* and *cmlA* leader peptides is 23S rRNA, it is worth considering the potential consequences on ribosome activity when a ribosome translates codons specifying a highly basic peptide such as polyarginine. Addition of polyarginine or polylysine to phenol-extracted rRNA produces a precipitate (111), as would be expected from the charges of the two reactants. If a nascent peptide has reasonably free access to the rRNA of its translating ribosome, a suggestion supported by the proposed mechanism of action of the *cat* and *cmlA* leader peptides, a highly basic peptide might perturb ribosome activity by causing conformational changes in rRNA. This reasoning predicts that translation of mRNA encoding a highly basic peptide might impede translation and function as a simple, charge-based pause signal. Two observations suggest that this interpretation may be correct. First, we have observed that the addition of polyarginine or polylysine (5- and 20-mers at 2 mM) to ribosomes completely

TABLE 1. Peptides encoded by several bacterial leaders or eukaryotic uORFs

Peptide	Source	Reference(s)
MVKTD	Codons 1–5 of *catA86* leader	29
MKKAD	Codons 1–5 of *catA194* leader	59
MKKSE	Codons 1–5 of *catA112/221* leader	8, 118
MSTSKNAD	Codons 1–8 of *cmlA* leader	125
KWFNID	Codons 11–16 of *tnaC*	40
MEVLALLRCFSSFFLLRLSSIRMPLVRRFTRHRLMISR	Codons 1–38 of *Lc* uORF	17
MQPLVLSAKKLSSLLTCKYIPP	Codons 1–22 of CMV uORF	20
MKLPGVRPRPAAPRRRCTR	Codons 1–19 of β_2-adrenergic receptor uORF	102
MNGRPSVFTSQDYLSDHLWRALNA	Codons 1–24 of *arg-2* uORF	31
MFSLSNSQYTCQDYISDHIWKTSSH	Codons 1–25 of *CPA1* uORF	139
MAGDIS	Codons 1–6 of AdoMetDC uORF	57

inhibits the peptidyltransferase reaction (111). Second, an unsuccessful effort by Gerchman et al. (38) to express chicken linker histone H5 in *E. coli* was traced to Arg-rich stretches in the protein. Codon bias and mRNA structure were ruled out as contributing factors. The current view is that protein domains rich in Arg were impeding translation, perhaps through an interaction of the protein with rRNA.

The category of peptides that alter ribosome function in the absence of other molecules includes certain peptide antibiotics that block protein synthesis (67). These inhibitory peptides are synthesized on ribosomes, but there seem to be no studies of possible *cis* effects of nascent forms of the antibiotic peptides on translation.

Evidence has recently been provided suggesting that an ORF in *E. coli* 23S rRNA may encode a peptide of five residues which, when expressed at high levels, confers erythromycin resistance on host *E. coli* cells (128). Translation in vitro of the ORF generates peptide that remains associated with ribosomes through sucrose gradient centrifugation. A synthetic version of the predicted peptide has been shown to interact with ribosomes in vitro in the absence of other cell components. Clearly, these observations become highly relevant to the biology of *E. coli* if rRNA can be a template for translation. While it has been shown that in vivo translation of a region of 16S rRNA is possible (4), it remains unclear whether portions of rRNA are normally available as template for translation.

Peptides as Coeffectors

cat and *cmlA* induction requires an additional component, chloramphenicol. Use of the alternative inducer amicetin has established a correlation between the amino acid sequence of the *catA86* leader-encoded peptide and the nature of the antibiotic that can serve as the inducer. Therefore, the leader peptide for *cat* and *cmlA* probably plays two roles in the inducible regulation. In one capacity, the leader peptide acts directly on the ribosome to pause elongation at a fixed location. In its second role, the peptide acts in concert with an antibiotic (the inducer) to stably stall a ribosome at the leader induction site. The nature of the antibiotic-dependent step is unknown, but perhaps the regulatory peptide alters the interaction of the antibiotic inducer with the ribosome, causing a different response from that seen in the absence of the peptide.

The regulation of the tryptophanase operon of *E. coli*, the *CPA1* gene of *S. cerevisiae* and the *arg-2* gene of *N. crassa* depends both on a sequence of amino acids in the uORF-encoded peptide and on the presence of a specific small molecule; tryptophanase is induced by tryptophan, and *CPA1* and *arg-2* are repressed by arginine. One might speculate that if the role of the *CPA1* and *arg-2* uORF-encoded peptides were to block ribosome release at the uORF stop codon, this activity could require the participation of arginine. Arginine is a basic amino acid and might cooperate with the leader peptide in altering the rRNA conformation to effect a block in translation termination. In contrast, the role that tryptophan plays in the induction of the tryptophanase operon remains unclear.

In a recent study of the conjugation response of *Enterococcus faecalis* to pheromone, *cis* regulation of translation is suggested as an explanation for inhibition of downstream expression of *prg*, a gene that encodes iCF10, the competence-inhibiting peptide (78). If this interpretation proves correct, the nascent peptide may function only with other factors of the complex pheromone response system.

An additional example in which the interaction between a peptide and a second component may alter ribosome function in *cis* is suggested by analyses of T4 exclusion by an integrated e14 prophage in *E. coli* (148). e14 encodes a protease (Lit) that is activated by a *gol*-encoded product (*gol* is a region within the major head protein gene of T4). When both *lit* and *gol* are overexpressed on plasmids, the Lit-Gol complex cleaves elongation factor Tu (EF-Tu) in *trans*. If the *gol*-encoded peptide is the activator of the protease, it is conceivable that upon the translation of this activator peptide (Gol), the peptide plus the protease might preferentially cleave EF-Tu in *cis*, affecting the function of the ribosome involved in translation of *gol* mRNA.

POSSIBLE MECHANISMS PREVENTING PEPTIDE EFFECTS IN *trans*

Expression of the *cat* leader at high copy number does not detectably interfere with cell growth. Accordingly, one or more of the following mechanisms may ensure that *cis*-acting regulatory peptides do not exert a *trans* effect on the general ribosome population of cells. First, peptides such as MVKTD and MSTSKNAD exhibit a low specific inhibition when added to ribosomes in vitro (42, 47). Since it is difficult to overexpress the peptides to the very high levels required for *trans* effects (1 to 2 mM), this alone could mandate that in vivo peptide effects will be limited to ribosomes directly involved in synthesis of the peptides. Second, regulatory peptides could be highly labile within cells, perhaps being destroyed immediately after exiting the ribosomes. Third, the general population of translating ribosomes in cells could be highly resistant to a *trans* effect by the inhibitor peptides, since the target for an inhibitor peptide in a translating ribosome should already be occupied by a peptide product of translation. Fourth, it is known that the *cmlA* inhibitor peptide is the product of the first 8 codons of a 9-codon leader and that the 9-mer peptide is not inhibitory (42, 51). Thus, other examples may exist in which the peptide that is released from the ribosome is a noninhibitory variant of the

inhibitor peptide as a result of the addition of one or more C-terminal amino acids. Lastly, it is conceivable that in certain instances a regulatory peptide may exert an effect on ribosomes only while the peptide remains attached to peptidyl-tRNA. For instance, the tRNA-peptide complex might effect a change in the ribosome that the peptide itself cannot.

At present, only one of the *cis*-active regulatory peptides appears to be generally toxic for cells when expressed at high levels; overexpression of *tnaC* in *E. coli* slows the growth of the cells (40). If the encoded peptide interferes with the action of the termination protein Rho, as is currently suspected, this could account for its toxicity. Apparently, at high levels the *tna* leader peptide overcomes the safeguards that normally prevent a *trans* effect.

ORIGIN OF PEPTIDES THAT INTERACT WITH THE RIBOSOME

It has been speculated that genes encoding ribosomal proteins could be the origin of leader sequences specifying the regulatory peptides (42), although there are no obvious homology relationships to support such a contention. On the other hand, peptides that *cis* regulate ribosomes are relatively short and it is conceivable that the coding sequence changes necessary to allow the peptides to perform specific regulatory functions may have obscured ancestral relationships. By contrast, the *cmlA* leader peptide (MSTSKNAD) does share apparent amino acid sequence similarity with microcin C7 (33, 48, 67), a peptide antibiotic known to inhibit bacterial protein synthesis in vivo and in vitro. Thus, certain of the *cis*-acting regulatory peptides may be ancestrally related to *trans*-acting peptide antibiotics that act on the ribosome.

OUTLOOK

Peptides that *cis* regulate ribosome activity in vivo have been identified because of their role in influencing downstream translation or, in the case of the *tna* operon, downstream transcription. In most examples, the regulatory peptide is encoded by a short ORF located immediately upstream of the regulated gene. In the translation attenuation system that regulates inducible chloramphenicol resistance genes, the regulatory peptides bind rRNA with targets in discrete domains of large-subunit rRNA. The rRNA-binding activity is believed to be responsible for the properties of the peptides as inhibitors of peptidyltransferase and translation termination; both activities are suspected of being catalyzed by a ribonucleoprotein in which the RNA is an essential component (97, 98, 132).

The notion that short peptides can modulate ribosome or rRNA activities is not a new concept. Certain peptide antibiotics which inhibit protein synthesis have specific rRNA targets (30, 112, 144). Furthermore, the cyclic peptide antibiotic tuberactinomycin can accelerate a ribozyme-catalyzed reaction (99), indicating that peptide effects on RNA can be stimulatory as well as inhibitory (136).

Detailed studies of the known systems of *cis* regulation by a nascent peptide will probably uncover distinct mechanisms through which various peptides can influence the activities of ribosomes; the action of the *tnaC*-encoded peptide is likely to differ from that of peptides specified by the leader of *catA86* or the uORF of *CPA1*. Identifying the function of each leader or uORF-encoded peptide and correlating that function with an amino acid sequence might be of use in identifying functionally analogous sequences embedded within long coding sequences. As an example, the *cat* and *cmlA* leader peptides appear to pause translation. During the expression of a long mRNA coding sequence, translational pausing could be important for the correct folding of the nascent protein product. Pause sequences in the nascent protein might serve as spacers separating folding domains of a protein, if correct folding could take place only in the (temporary) absence of downstream amino acid sequences. Indeed, examples of translational pausing or arrest exist (84, 140, 142), and cases may be identified in which the regulation depends on the nascent peptide being translated at the time of the pause.

It is operationally difficult to identify pause sequences within genes. An alternative is to screen protein databases for the occurrence of the known pause sequences such as MVKTD, MSTSKNAD, MKKAD, and MKKSE. As an example, MKKAD represents the first 5 amino acids of the leader peptide encoded by *catA194* (59) and MKKAD occurs at an internal site in the C5a protein secreted by *Streptococcus pyogenes* (13). Whether MKKAD functions as a pause sequence in the context of the amino acids that precede MKKAD in the C5a protein is currently unknown, although the available technology allows the testing of this and other examples.

Nascent peptide effects on ribosomes probably extend beyond serving only as signals for ribosome pausing or ribosome arrest at a stop codon (see, e.g., reference 138). It will therefore be valuable to determine the mechanism of action of the nascent peptides in apparently simple systems such as *cat* regulation and inhibition of gp48 translation and in seemingly more complex processes such as arginine-mediated repression of *CPA1* and *arg-2* translation and ribosome hopping in the T4 topoisomerase gene. These studies may reveal fundemental control circuits essential for the correct translation of mRNAs.

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